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THE EFFECT OF INJECTIONS OF HEMOLYTIC STREPTOCOCCI ON SUSCEPTIBLE AND INSUSCEPTIBLE ANIMALS.

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The observations recorded in this paper were made in the course of a study of streptococcus infection and immunity. In order to study the problem of the immunization of animals against streptococcus infection, it was necessary first to obtain as clear a picture as possible of the course of the infection in untreated animals. It has been noted by other workers, and is borne out by our own observations, that bacteria introduced into the circulation of the living animals quickly disappear. It was our first task to study this phenomenon and if possible to find some means of explaining it.

We found that with hemolytic streptococci, which are of rather low virulence for rabbits, a sublethal dose may completely disappear from the blood stream within a few hours. If a lethal injection is given, over 90 per cent of the cocci are removed from the circulation within the first few minutes, and subsequent blood cultures reach a minimum in 2 to 3 hours, but after 4 to 6 hours the number again begins to increase. Even with lethal doses, cultures of 1 cc. of blood may show no colonies after 2 or 3 hours, but, as a rule, streptococci do not completely disappear after such an injection. The usual course of a primary removal is shown by the cultures taken after the first injection recorded in Experiment 2. An example of complete removal is shown in Experiment 1.

Experiment 1.—Rabbit injected with 5 cc. of a heavy suspension of Streptococcus 43. Cultures taken immediately showed several thousand organisms per cc. of blood. After 10 minutes, five drops of blood showed only 23, and after 30 minutes, 5; after 1 hour, 2 hours, $4\frac{1}{2}$ hours, $11\frac{1}{2}$ hours, and 26 hours, 1, 0, 6, 17, and 118 organisms respectively.

The blood cultures subsequent to those recorded in the experiment were consistently positive until the death of the animal on the 4th day. The prompt and almost invariable removal of organisms artificially introduced into the circulation has been difficult to harmonize with the fact that in susceptible animals the organisms again appear in the blood within 4 to 12 hours, and may be found constantly in the circulation until death, as in cases of spontaneous septicemia.

There are four hypotheses which might account for this: first, that after a period the blood-sterilizing mechanism becomes exhausted and permits bacteria to remain in the circulation; second, that the bacteria after a brief stay within the body become resistant to its defensive powers; third, that the injection of foreign protein stimulates some reaction such as a mobilization of antibodies, or of phagocytes, which would account for the prompt and extensive preliminary removal of the bacteria; fourth, that the bacteria do not multiply in the circulation, but are continually introduced from infected tissues faster than they can be removed.

The first possibility is practically ruled out by the fact that if, during the period of the reappearance of bacteria from the circulation, an additional number many times in excess of those present is introduced, they are removed as rapidly, or even more rapidly than after the first injection, and that this reinjection, and subsequent removal of the bacteria, may be repeated until the animal becomes moribund. This fact, originally observed by Bull,[1] is well illustrated in the following experiment:

Experiment 2. Removal of Streptococci after Repeated Injections.—A rabbit weighing 1,620 gm. was injected into the ear vein with a suspension of Streptococcus 5, containing 72,000 chains of small clumps per cmm. Five drops of blood from the opposite ear were plated in sheep blood agar at the intervals noted.

First Injection, 4.8 Cc.—Cultures taken after 1 minute showed 60,000 colonies; sank to 135, 3, and 5 after 10 minutes, 1 hour, and 4 hours respectively; rose to 1,800 at 6 hours; showed 127, 150, and 8 colonies after 23 hours, 25 hours, and 25 hours, 4 minutes respectively.

Second Injection, 25¼ Hours after First Injection. 2.4 Cc. Injected.—Cultures ½ minute after injection showed 62,000 colonies; sank to 19 after 10 minutes and remained low for 3 hours; after 4 hours cultures showed 200 colonies; after 7½ hours, 100, after 8 hours, 56.

[1] Bull, C. G., *J. Exp. Med.*, 1916, xxiv, 7.

Third Injection, 8 Hours, 10 Minutes after Second Injection. 0.5 Cc. Injected.—Cultures ½ minute after injection showed 21,000 colonies; fell to 15 after 10 minutes; after 20 minutes, ½ hour, 1½ hours, 2½ hours, and 13½ hours, showed 180, 280, 75, 30, and 220 colonies, respectively; rose to 1,320 at 16½ hours; and after 20 hours, 21 hours, 23 hours, 11 minutes, and 23 hours, 27 minutes, showed 33, 51, 120, and 221 colonies, respectively.

Fourth Injection, 24 Hours, 28 Minutes after Third Injection. 4.9 Cc. Injected.—Cultures ½ minute after injection showed 65,000 colonies; sank to 90 after 11 minutes, and remained fairly low for 3½ hours; rose to 480, 4½ hours after injection. Died 20 hours after injection.

The second possibility, namely, that the streptococci have become resistant, deserves more careful observation. Considerable evidence has been brought forward to show that bacteria which have grown in the body differ strikingly in their resistance from the same organisms cultivated artificially. The studies of Gruber and Futaki[2] on anthrax, and of Bail,[3] Eisenberg,[4] and others on Gram-negative bacilli, are too familiar to require more than passing reference. In his studies on pneumococcus infection in dogs, Bull[1] has shown that the organisms which reappear in the circulation after the primary fall in sepsis are more resistant to the action of immune sera than those injected.

However, in the case of hemolytic streptococcus infection in the rabbit, this does not seem to offer a satisfactory explanation. It is true that streptococci which have multiplied in the peritoneal cavity of a mouse show a striking resistance to agglutination and phagocytosis, even in the presence of immune serum derived from rabbits, as compared with streptococci from culture. The latter, as a rule, agglutinate spontaneously, and are extensively phagocyted in salt solution, whereas suspensions from the peritoneums of infected mice have often failed to agglutinate in the immune sera in our possession, and show high relative resistance to phagocytosis. Nevertheless, if these bacilli from the mouse peritoneum are injected into a rabbit's circulation, they are disposed of almost as effectually as are culture organisms. This is illustrated in the following experiment:

[2] Gruber, M., and Futaki, K., *Münch. med. Woch.*, 1906, liii, 249.

[3] Bail, O., *Arch. Hyg.*, 1905, lii, 272.

[4] Eisenberg, P., *Centr. Bakteriol., 1te Abt., Orig.*, 1908, xlv, 638.

Experiment 3. Removal of Streptococci Obtained from Peritoneum of Mouse.—Three mice were injected intraperitoneally with Streptococcus 5. The 24 hour exudate was removed in citrate, centrifuged to remove leukocytes, then more rapidly to throw down the cocci. The second sediment was suspended in salt solution. A broth culture of the same organism was centrifuged and the sediment resuspended in salt solution. Two rabbits were injected into the ear vein and cultures of fifteen drops taken from the opposite ear. It is difficult to count accurately the number of organisms in the suspensions, and it is possible that the doses were unequal. Nevertheless, the prompt removal of nearly all the streptococci from the circulation is evident, though it would appear that the culture organisms were removed more rapidly than those from the mouse.

Rabbit 1, weight 1,010 gm., was given 10 cc. of streptococcus suspension from a mouse, containing 64,000 chains per cmm. Cultures at 5 minutes showed 2,000,000 colonies; at 10 minutes, 1,300; at 30 minutes, 900; at 1 hour, 103; at $3\frac{3}{4}$ hours, 257.

Rabbit 2, weight 980 gm., was given 10 cc. of streptococcus suspension from a culture containing 56,000 chains per cmm. Cultures at 5 minutes showed 1,000,000 colonies; at 10 minutes, 400; at 30 minutes, 60; at 1 hour, 24; at $3\frac{3}{4}$ hours, 192.

This experiment is open to the objection that the resistance acquired by streptococci growing in the peritoneum of a mouse might not be the same in degree or in kind, as that acquired in the circulation of the rabbit. It is technically impossible to introduce into a rabbit's circulation bacteria from the circulation of another rabbit comparable in number to those which can be introduced from cultures. However, the following experiment seems to show that a change in the streptococci during their multiplication in the animal is not the factor which determines their persistence in the circulation. A rabbit was given a large intravenous injection of organisms from cultures, and 48 hours later, when the organisms were numerous in the blood, and the animal was almost moribund, 20 cc. of blood were taken from the heart, under ether, into a cooled paraffined syringe and immediately injected into the ear vein of a normal rabbit. The blood used for injection contained 6,600 organisms per cc. Cultures taken from the second rabbit gave the following result:

Experiment 4.—Cultures taken from the second rabbit showed 2,400 per cc. 1 minute after injection; 600 after 3 minutes; sinking gradually to 108 at 2 hours; at 24 hours, 16 colonies; and at 48 hours, 0. The animal survived.

This seems to show that the streptococci which one finds in a so called septicemic rabbit have not acquired resistance sufficient to maintain them in the circulation.

In regard to the third possibility, a number of our earlier observations seemed to indicate that the injection of the bacteria, acting as a foreign protein, excited some reaction in the animal which caused their removal. On several occasions when a septicemic animal was reinjected with the same organisms, the bacterial count 1 or 2 hours after injection fell to a point distinctly below the count before injection. This seemed to indicate that, following the second dose, the animal got rid of not only all the newly introduced organisms, but also some of those previously in the circulation. Bull[1] has reported this same phenomenon in dogs injected with pneumococcus.

Experiment 5. Reduction in Sepsis Following Reinjection. First Injection. 3 Cc. Injected.—3 cc. of streptococci containing 55 million per cc. injected. After 1 minute culture of fifteen drops from opposite ear showed 22,000 colonies; fell to 95, 12, 2, 9, and 3, after 10 minutes, 30 minutes, 1 hour, $3\frac{1}{2}$ hours, and $4\frac{1}{2}$ hours after injection respectively.

Second Injection, 4 Hours, 20 Minutes after First Injection. 3 Cc. Injected.—Cultures showed 23,000, 1 minute after injection; 8, 2, and 936, 10 minutes, 43 minutes, and 18 hours after injection respectively.

Third Injection, 18 Hours, 20 Minutes after Second Injection. 5 Cc. Injected.—Cultures showed 3,000 colonies 1 minute after injection; 38, 32, 52, and 750, 10 minutes, 45 minutes, $2\frac{3}{4}$ hours, and 6 hours after injection respectively.

It will be noted that following the third dose, the greater number of the injected organisms was removed within 1 minute, as the first culture taken contained only 3,000 colonies as compared with 22,000 and 23,000 following the first and second injections. In 10 minutes after the third injection the count dropped to 38 as compared with 936 before the injection. In other words, not only did the organisms injected disappear from the blood stream, but the majority of those previously present in the circulation also disappeared, and it was not until more than $2\frac{3}{4}$ hours after the third injection that the sepsis again approached its previous intensity. A similar result was obtained following the second injection of Rabbit 4, Table I, and following the third injection recorded in Experiment 2.

The result could not be obtained regularly in experiments conducted in a similar way, but occurred with sufficient frequency to make necessary further investigation. As an explanation of this reaction there occurred to us the possibility that the injection of the additional number of streptococci caused a mobilization of antibodies, or, more probably, a sudden leukocytic reaction such as has been noted following the injection of specific and also of non-specific proteins into infected animals.

It seemed possible that in cases where the count after injection did not fall below that before injection the removal of the organisms already present in the blood might be masked by the large additional number injected. If the removal of the organisms from the blood following a second injection was due to a reaction by the body to the injected streptococci, acting as a foreign protein, it was probable that it could be reproduced by the injection of killed streptococci, of other species of bacteria, of serum, or of peptone. Should it occur after injections of such non-specific substances, it would appear more clearly, as the picture would not be confused by the growth of the newly injected organisms in cultures taken after injection. However, in a series of experiments in which the substances mentioned above were injected into septicemic rabbits, no consistent effect on the number of organisms in the circulation could be observed.

On further study it appeared that we had at first attached too great significance to these apparent remissions in the sepsis following reinjection. It will be noted in Experiment 2, that after the third injection the number of organisms in the circulating blood varied enormously within a few hours. These variations have been noted so often that we believe the possibility of their being due to technical error can be ruled out. The only influences brought to bear upon the animal during this period of variation were the withdrawal of small samples of blood from the ear and placing the animal head down in a warmed box to facilitate bleeding. We have carefully tested the effect on septicemic animals of repeated small bleedings, of maintenance for considerable periods in a vertical position, and of maintenance in a warm chamber, but none of these procedures had any constant effect on the course of the sepsis. As our observations accumulated, these variations continued to appear and to support

the idea, which we shall refer to again, that streptococcus rabbit sepsis is not a condition in which a definite number of organisms is constantly in the blood, but one in which organisms are continually swept into the circulation from heavily infected tissues.

The possibility that a leukocytosis was responsible for the removal of the organisms from the blood was definitely excluded by making blood counts. The effect of streptococcus injections on the leukocytes was not constant, but the usual reaction was an immediate leukopenia, sometimes rather marked, as illustrated by Rabbit 3, Table I. Repeated injections as a rule caused a still further fall of leukocytes, as illustrated by Rabbit 4, Table I. In a few instances after smaller injections, a transitory rise in the blood count, as illustrated in Rabbit 5, Table I, was observed, but this was exceptional and could not account for the removal of the injected bacteria which was invariable. Whether or not the leukopenia is usually followed by a leukocytosis we cannot state. In several animals studied we failed to observe it. We did not, however, make frequent counts for more than 6 hours after injection, as by this time the streptococci have not only been removed from the circulation, but, as will be seen later, have been largely killed, and a leukocytosis occurring later could have no bearing on the phenomena we were endeavoring to

TABLE I.

Rabbit 3. Leukopenia Following First Injection. 3 Cc. of Streptococcus Suspension.

Time.	Temperature.	White blood count.	Colonies per cc.
	°F.		
Before injection	102.8	9,000	
1 min. after	102.9	6,100	1,000
10 " "	103.0		27
½ hr. "	103.4	5,800	7
2½ hrs. "	105.4	3,100	
4 " "	105.8	2,600	4
1 day "	105.0	4,100	500
2 days "	105.4	6,100	43
3 " "	103.6	6,000	0
4 " "	105.4	9,400	0
5 " "	104.5	9,100	0
7 " "	105.0	15,000	1,170

TABLE I—*Concluded.*

Rabbit 4. Progressive Leukopenia Following Second Injection.

Time.	Temperature.	White blood count.	Colonies. per cc.
	°*F.*		
Before injection		10,800	
1 min. after 1st injection	102.6		1,000,000
10 " " 1st "		6,500	1,820
30 " " 1st "	105.1	8,200	37
1 hr. " 1st "	105.7	6,200	35
2½ hrs. " 1st "	104.2	7,200	
4½ " " 1st "	105.6	5,200	169
4 " 35 min. Reinjection.			
1 min. after 2nd injection			900,000
10 " " 2nd "			569
30 " " 2nd "	105.6	3,100	48
1 hr. " 2nd "	104.7	2,800	194
2 hrs. " 2nd "	103.6	500	214

Found dead 21 hours after 1st injection.

Rabbit 5. Transitory Leukocytosis Following Injection.

Time.	Temperature.	White blood count.	Colonies. per cc.
	°*F.*		
Before injection		6,800	
1 min. after			13,000
30 " "	101.9	11,400	336
2½ hrs. "	103.8	7,600	34
5 " "	103.6	4,700	315
12 " "		3,500	600

Found dead 22 hours after injection.

explain. The absence of a general leukocytosis does not, of course, exclude the possibility that in the visceral capillaries the leukocytes take part in the destruction of the invaders. This question will be taken up later.

To repeat, the injection of streptococci is not immediately followed by a leukocytosis and we have been unable by the injection of various non-specific substances to excite any reaction which would remove the bacteria from the circulation of a septic animal. These negative results do not exclude the possibility that the living streptococcus

itself can cause some such reaction. However, in view of the fact that the injection of additional streptococci does not regularly cause a reduction in the sepsis, and also of the fact that the reductions we have observed have all been within the range of spontaneous variations in such infections, we believe that a reaction of this sort can be reasonably excluded as an explanation for the primary removal of bacteria from the blood stream.

The fourth possibility, that the bacteria found in the blood of septicemic rabbits are not actively persisting in the circulation, but are merely swept into the circulation from infected tissues more rapidly than they can be removed seems to us the most probable explanation. This hypothesis has been commonly adopted for infections such as subacute bacterial endocarditis in man, where the septicemia is intermittent. While at first sight this supposition seems unlikely in the case of a continuous sepsis, the fact that in these animals the number of bacteria in the muscles and other organs far exceeds those in the blood, weight for weight, would at least explain the source of the constant supply of bacteria. We shall discuss this point more fully later.[5]

Mechanism of Removal.

The observations mentioned above show that even a susceptible animal, such as the rabbit, is able to remove from its circulation almost any number of introduced streptococci, even after successive injections and until a lethal condition has developed, and will almost or completely free its circulation from the organisms within a few hours unless a new supply is reintroduced. Concerning the mechanism of this removal and the ultimate fate of the bacteria, however, there has been considerable discussion.

Three factors have been suggested as entering into this removal of bacteria: agglutination, phagocytosis by leukocytes, and phagocytosis by fixed cells. We have looked for evidence, such as that reported by Bull, of agglutination in rabbits and in cats. The organisms as injected, even though previously shaken for a long time with glass beads, often show clumps of from two to ten chains. Clumps of this

[5] We are indebted to Miss B. H. Paige for assistance in the blood culture studies.

size have naturally been found in the blood and in the organs, but seldom have they exceeded the diameter of the average capillary. We have also seen groups of streptococci of about the size and contour of a leukocyte sometimes entangled with leukocytic remnants. These we have interpreted as being groups of organisms which had been taken up by leukocytes which have subsequently disintegrated. Aside from these small groups of cocci which do not resemble in the least the felt-like masses of streptococci which have agglutinated *in vitro* we have seen neither in films of the blood nor in crushed preparations or sections of the organs evidence of agglutination, and it seems unlikely that it plays a part in the phenomenon we have studied.

In regard to the leukocytes, these cells undoubtedly take up injected streptococci in considerable numbers, and with great rapidity, as can be seen a few minutes after injection in blood films or in sections, and it has been frequently claimed that these cells play a predominating part in the removal of microorganisms from the blood. Wyssokowitsch,[6] however, found the bacteria lying apparently in endothelial cells, and recently Kyes[7] has demonstrated in the pigeon the taking up of pneumococci by the Kupffer cells of the liver, and by endothelium in other organs of the body. Manwaring and Coe[8] have analyzed this phenomenon further by carefully washing livers free from blood and then perfusing the organs with virulent pneumococci and other organisms. They found that whereas virulent pneumococci treated with immune serum were quantitatively removed by the blood-free liver, these organisms when suspended in Ringer's solution or in normal serum were not removed even by livers of immunized animals, or were taken up to a slight extent. This they attributed to the action of traces of serum which had not been removed from the organs, and they speak of the immune substance to which this is due as "endothelial opsonins."

Bartlett and Ozaki,[9] in studies on dogs injected with staphylococci, noted the primary accumulation in the lungs, but describe the leukocytes as taking up the majority of the cocci.

However, the original observations of Wyssokowitsch[6] point clearly to the importance of phagocytosis by endothelial cells in the removal of bacteria from the circulating blood. He says: The defensive power of the body lies much more in the structures of the vessel walls; that is, in the endothelial cells of the blood vessels. The bacteria which reach the blood stream are deposited in or between the endothelial cells, which line the capillaries most numerously in organs where

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[6] Wyssokowitsch, *Z. Hyg.*, 1886, i, 3.

[7] Kyes, P., *J. Infect. Dis.*, 1916, xviii, 277.

[8] Manwaring, W. H., and Coe, H. C., *J. Immunol.*, 1916, i, 401.

[9] Bartlett, C. J., and Ozaki, Y., *J. Med. Research*, 1916–17, xxx, 465.

the blood stream is slowest. It is here that the battle between the cells and bacteria concerning which we have so much evidence from many sides takes place. However, we still have no decisive knowledge of the course of this conflict, nor of the means of attack and defense.

In sections from the organs of recently injected animals it is difficult to determine how the streptococci are taken up. We have studied chiefly the lung, as this is the organ most concerned in their removal. In lung sections of animals killed 10 to 30 minutes after injection it is clear that some of the cocci are held within the leukocytes in the capillaries. It is equally clear that the majority are not located within leukocytes. Many of them lie in large mononuclear cells which are probably the swollen endothelium of the capillaries; others lie in an eosin-staining matrix which may represent a section through endothelial protoplasm, or possibly some substance from the blood deposited about them.

In order to study this point more accurately, we have etherized and killed cats within 30 minutes after injection, washed the lungs as free as possible by perfusion with salt solution, and then fixed them by injecting Helly's fluid under moderate pressure in order to keep the capillaries distended. In sections fixed in this way the streptococci are not washed from the capillaries; even leukocytes are still present in moderate numbers. Of the mechanism by which the cocci are held, however, we are still in doubt, though many appear to be in endothelial cells.[10]

Fate of the Phagocyted Cocci.

More important for the understanding of the resistance to streptococcic infection than the mechanism of removal of the organisms from the circulation seems to be the means by which they are ultimately destroyed.

The possibility that the body freed itself by excreting the living bacteria was considered by Wyssokowitsch[6] and ruled out on account of the small number which he could recover from the urine, intestinal contents, or milk. Our own observations on the urine and bile of infected rabbits made clear that few living streptococci were passed

[10] We wish to acknowledge our indebtedness to Prof. W. G. MacCallum for his advice and cooperation in these experiments.

out in these secretions. It seemed then that there must be within the body some mechanism for their destruction. In the rabbit this process is obscured by the fact that after a period of apparent destruction the bacteria in the blood stream again begin to multiply. The cat, however, is practically insusceptible to artificial streptococcus infection and large numbers of organisms introduced into the blood are promptly removed as in the rabbit and do not again reappear, or at least reappear only for a brief period.

It has been seen that the streptococci which disappear from the blood are taken up in part by the leukocytes but probably more extensively by the endothelial cells, at least the majority are held in the viscera by some mechanism other than ingestion by polynuclears. The fate of the organisms taken up in these two ways was studied, as far as possible separately.

Bactericidal Effect of Leukocytes.

There is no doubt, as stated above, that a considerable number of cocci is taken up by the leukocytes, but the fate of the organisms so taken up is by no means clear. Schattenfroh,[11] Pettersson,[12] Kling,[13] Zinsser,[14] and others have shown that extracts of leukocytes have distinct bactericidal action on many species of organisms, although Watabiki[15] has failed to demonstrate this action. However, when whole living leukocytes have been tested, Pettersson, Kling, and most others who have investigated their action have failed to demonstrate bactericidal effect.

Our results have not been entirely clean-cut. We have used both whole clotted blood and cells from citrated blood. The technique for clotted blood was as follows: An animal was bled shortly after injection and 1 cc. of blood was immediately measured into each of a series of test-tubes. One sample was emptied into a mortar as soon as clotted, ground with sand, and poured into melted agar. The test-tube and mortar were rinsed with saline solution, the wash was added

[11] Schattenfroh, A., *Arch. Hyg.*, 1897, xxxi, 1.

[12] Pettersson, A., *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 498.

[13] Kling, C. A., *Z. Immunitätsforsch., Orig.*, 1910, vii, 1.

[14] Zinsser, H., *J. Med. Research*, 1910, xxii, 397.

[15] Watabiki, T., *J. Infect. Dis.*, 1909, vi, 319.

to the agar, and the whole plated. Other tubes were plated in this way after various periods of incubation. In one instance there appeared to be a distinct reduction in the blood of a normal cat (Table III). In normal or immune rabbits there was a slight reduction or a gradual increase. The method is not sufficiently accurate to enable one to attach significance to these irregular results. It is impossible to obtain separate colonies from all the streptococci from clotted blood, as is shown by the fact that the count from blood ground immediately after coagulation is much lower than that before coagulation.

On account of the unreliability of this method we made other experiments in which the animal was bled into citrate solution, and the cells were thrown down in the centrifuge, washed once in saline solution, and resuspended in serum or in saline solution. They were then inoculated with a small amount of streptococcus suspension and the measured amount was plated at various intervals. The result of an experiment with normal cat cells and serum is shown in Experiment 6.

Experiment 6.—0.1 cc. of 1.25 cc. of cells plus 1.25 cc. of serum showed 550 colonies immediately, 520 after 1 hour, and 609, 750, and 2,700 after 2, 4, and 8 hours respectively. 0.1 cc. of 1.25 cc. of cells plus 1.25 cc. of salt solution gave 500 colonies immediately; 400, 450, 600, and 3,000 after 1, 2, 4, and 8 hours respectively. 0.1 cc. of 2.5 cc. of serum alone gave 280 colonies immediately; 300, 170, 170, and 3,000 after 1, 2, 4, and 8 hours respectively. 0.1 cc. of 2.5 cc. of salt solution alone gave 42 colonies immediately; 0, 0, 1, and 0 colonies after 1, 2, 4, and 8 hours respectively.

Control tubes of blood cells in serum and blood cells in salt solution were inoculated with a large number of streptococci for morphological study. Films made after 1 hour's incubation showed extensive phagocytosis both in serum and salt solution, but more marked in the serum tubes.

The only tube in which there was a distinct destruction of the streptococci was in the salt solution control. Other experiments in which normal or immune rabbit serum and cells were used gave similar results. In the tests made with citrated cells we never observed a reduction in the count comparable to the isolated result with clotted blood recorded in Table III. We are uncertain whether or not the slight reduction in the counts from the serum tube in the experiment

above, or similar reductions in tubes of serum and cells in other experiments indicate a feeble bactericidal action on the part of these elements. It seems certain, however, that if it exists it is much inferior to the destructive power of the lung tissue which we are about to discuss, and that it is entirely insufficient to account for the extensive destruction of the injected bacteria which takes place. It seems unlikely from these experiments that the leukocytes can even destroy the small shares of these organisms which they can be seen to ingest.

Bactericidal Effect of the Tissues.

In contrast to this doubtful evidence that the leukocytes or serum can take part in the destruction of invading streptococci, it can be clearly shown that certain tissues, especially the lung, are possessed of marked bactericidal power. The most convincing evidence is that obtained from cats killed at various intervals after injection.

If a cat is killed within a half hour after receiving streptococci intravenously, cultures show an enormous number of the cocci in the lungs, many in the liver and spleen, and in the muscles, blood, and kidneys a few or none at all, the result depending on the number injected (Tables II and IV). In a few experiments in which the bone marrow and lymph nodes were also examined these tissues contained far less than the spleen but rather more than the last mentioned tissues.

Cultures from a cat killed several hours after an injection show a smaller number of colonies from all the viscera, but perhaps a slightly increased number in the muscles. The decrease is most marked in the lung, but is also pronounced in the liver. The spleen seems to rid itself more slowly of the cocci. If the dose is small, all the tissues may be found sterile in 8 hours; if very large, positive cultures may be obtained for more than 24 hours. In the cats recorded in Table II the tissues were practically sterile in 24 hours. The most interesting point is that when films were made from the ground lung tissue at a stage when the cultures showed a greatly diminished number of viable bacteria, or none at all, numerous stainable streptococci were found. They were found up to the 5th day, which is as far as our observations have extended. In comparing Cats 1 and 4 it is seen

that, whereas in 5 days the viable bacteria had dropped from 300,000 to 0 per dg., the number of organisms seen in films seemed undiminished. This seems to admit of but one interpretation; namely, that the streptococci have been taken up in the lungs and killed, although they remained visible for a considerable period of time.

Fate of Streptococci in Cat Tissues.—Four cats were injected intravenously with 40 million streptococci per kilo and killed at the intervals noted. Pieces of the organs to be studied were placed in a sterile test-tube, weighed, ground in a sterile mortar with sand, and emulsified in salt solution added in the proportion of 1 cc. to 0.1 gm. of tissue. Plates were made in sheep blood agar of amounts of organ emulsion representing from 0.1 to 0.0001 gm. and the colonies counted after 24 hours. Films were made from the ground emulsion and stained by Gram's method. As previous experiments had shown most pronounced effect in the lung, the lung cultures were made in duplicate from different parts of the organ.

TABLE II.

Tissue.	Cat 1. Killed 10 min. after injection.			Cat 2. Killed 5 hrs. after injection.		Cat 3. Killed 24 hrs. after injection.		Cat 4. Killed 5 days after injection.	
	Film.	Colonies per 0.1 gm. Tissue ground and plated immediately.	Colonies per 0.1 gm. Tissue ground and plated after 5 hrs.' incubation.	Film.	Colonies per 0.1 gm.	Film.	Colonies per 0.1 gm.	Film.	Colonies per 0.1 gm.
Lung	---	300,000	160,000	++	800	+++	1	+++	0
		330,000	80,000		72		0		0
Spleen	+	18,000	140,000	0	1,000	0	1	0	0
Liver	+	34,000	44,000	0	43	0	0	0	0
Kidney		100	3,000*		3		0		0
Bone marrow	0	500			7		2		0
Psoas	0	8	52	0	20	0	0		0
Pectoralis		27	About 12,000		53		0		0
Blood		26	1,200		12		0		0

* 3,000 indicates that the colonies in a plate from 0.1 gm. were too numerous to estimate and that no plates were made from smaller amounts of tissue.

It seemed important to attempt to demonstrate this bactericidal action of tissue, which is so pronounced in the lung *in vitro*. In the

experiment in Table II pieces of the organs from the cat killed 10 minutes after injection were removed and distributed in sterile test-tubes which were placed in a moist chamber at 37°C. for 5 hours, in order to compare the action of the excised tissue with that of the tissue of the cat which had been allowed to live for the same period. At the end of this time the pieces were weighed, ground, and measured amounts planted as shown in Table II. It will be noted that in the living animal there was an enormous reduction in the number of organisms in the lung and liver and a less pronounced reduction in the spleen, bone marrow, and blood. The reduction in the last may well have been due to other factors than the bactericidal effect of the blood itself. On the other hand, there was an increase in the number of bacteria in the muscles during this period.

If these findings are compared with the fate of the bacteria in Cat 1 which was killed 10 minutes after injection, it will be noted that the lungs showed a reduction in the number of bacteria which was distinct though scarcely comparable with that taking place in the living

TABLE III.

Bactericidal Effect of Cat Tissues.

A normal cat weighing about 3 kilos was injected with 2 cc. of a streptococcus suspension containing 30 million per cc. In 10 minutes it was bled to death under ether and perfused for 10 minutes through the jugular to wash the blood from the lung. Pieces of the lung were placed in sterile test-tubes, and the animal was then perfused 10 minutes through the proximal portion of the carotid to remove the blood from the other tissues. Pieces of the other tissues were then distributed in sterile test-tubes. They were incubated, weighed, ground, and plated as in the preceding experiment.

Tissue.	Colonies per 0.1 gm. of tissue.				
	Plated immediately.	After 1 hr.'s incubation.	After 2 hrs.' incubation.	After 4 hrs.' incubation.	After 11 hrs.' incubation.
Lung	26,000	18,000	3,300	9,000	2,400
	26,000		750	2,000	300,000
Spleen	360		1,000	3,700	3,000
Liver	1,300		560	920	3,000
Kidney	0		8	32	54,000
Psoas	1		—	0	1,500
Blood	20		0.3	9.2	12

animal, whereas in the spleen, liver, and blood the organisms multiplied moderately, and in the kidney and muscle tissues multiplied with great speed. Further experiments showed that the destruction by excised tissue took place only during the first 2 or 3 hours of incubation, after which, owing probably to death of the cells, they again multiplied. This is illustrated in Table III.

TABLE IV.

Bactericidal Test of Cat and Rabbit Tissues.

A normal cat weighing 4,000 gm. was injected intravenously with 4 cc. of streptococcus suspension containing 400,000 per cc. (400,000 per kilo). It was killed with gas in 10 minutes, and the specimen removed and cultures were made as before.

A rabbit weighing 1,600 gm. was given 3.2 cc. of suspension containing 200,000 per cc. (400,000 per kilo). It was killed in 10 minutes and cultures were made as above.

Tissue.	Plated immediately.	After 1 hr.'s incubation.	After 2 hrs.' incubation.	After 3 hrs.' incubation.	After 4 hrs.' incubation.	After 6 hrs.' incubation.	After 10 hrs.' incubation.
			Cat.				
Lung 0.1 gm.	2,600	2,800	100	140	1,600	4,000	∞
" 0.1 "	3,700	2,100	720	1,200	105	5,200	
Spleen 0.1 "	6		2				∞
Psoas 0.1 "	0		0				0
Blood 1 cc.	0						
			Rabbit.				
Lung 0.1 gm.	750	720	710	3,000	19,000	∞	∞
" 0.1 "	1,400	750	1,200	4,500	17,000	∞	
Spleen 0.1 "	70		900				
Psoas 0.1 "	0		0				
Blood 1 cc.	0						

This experiment seems to show a definite decrease in the number of viable bacteria in the lung tissue when incubated. The action of the tissue was evidently much impaired by its removal from the body and after a few hours the bacteria multiplied freely in the dead tissue. The decrease in the lung is more striking if compared with the prompt increase in the spleen, or, in experiments with rabbits, in the muscles.

The process can be more definitely demonstrated if a much smaller injection is made; in fact it is difficult to compare the organs of the same animals *in vitro*, as, if the injection is small enough to show clearly the effect in the lung, there are not sufficient bacteria deposited in the other organs to give positive cultures from amounts which it is practicable to test.

Table IV records the results observed in a cat which was given a much smaller injection, so that the blood was completely sterilized in 10 minutes. In this experiment a rabbit was injected for comparison with a proportional dose of the same suspension. The difference in the distribution of the organisms and the effect of the tissues on them will be referred to later.

From the results recorded in the preceding experiments there seemed little doubt as to the killing of the streptococci in the lung. However, on account of the large differences between duplicate samples of lung tissue, during the later stages of these experiments it seemed best to repeat the experiment in quadruple. The results are recorded in Experiment 7, in which each entry represents the count from a piece of lung weighed, ground, and plated separately.

Experiment 7. Bactericidal Effect of Lung Tissue of Cat.—A normal cat was inoculated with 12 cc. of a streptococcus suspension containing 100 million per cc. The animal was killed with gas after 10 minutes. The autopsy was completed and first plants were made 30 minutes after injection.

0.1 gm. of lung tissue showed 550, 248, 200, and 480 colonies immediately; 34, 40, 28, and 60 colonies after 2 hours; and 120, 53, 25, and 65 colonies after 5 hours.

0.1 gm. of spleen tissue showed 146 colonies immediately; 194 after 2 hours; and 3,000 after 5 hours.

The reduction in the number of colonies obtained from the lung after incubation was so constant and so marked that, even though it varied in degree in different pieces of the same lung, it seemed certain that it was due to an extensive destruction of the streptococci which took place in the tissue *in vitro*. The question at once arose as to what element of the tissue was responsible for this destruction. The uncertainty as to the location of the cocci in the lung makes this difficult to answer. If, as appears from a study of the sections, the majority of the cocci are taken up by endothelium, it is most likely that these are the cells which destroy them. That the bactericidal

power lies in some of the fixed tissue elements and not in the blood appears certain from the following considerations: In the first place, the experiments described above show that the whole blood, the blood cells, and the blood serum are incapable of destroying any considerable number of streptococci. In the second place, the tests recorded in Table III were made with tissue that had been freed as much as possible from blood and from free lying cocci by perfusion before the pieces were excised for examination. Moreover, in order to rule out participation of the elements of the blood in the taking up and destruction of the streptococci we performed the following experiment:

Experiment 8.—A cat was first perfused for from 2 to 3 hours with citrate and Ringer's solution, and then perfused with a very dilute streptococcus suspension (about 400 organisms per cc.). The lung was again perfused with sterile Ringer's solution until the content of the outflow was reduced to 35 per cc. Pieces of lung were then removed and treated as in the previous experiments.

Culture from 0.1 gm. of perfused lung gave 250 and 170 colonies immediately; 42 and 3 after 2 hours; 16 and 0 after 4 hours; 0 colonies after 7 and 24 hours.

In view of the fact previously mentioned, that streptococci quickly die when placed in a dilute suspension in salt solution, the possibility must be admitted that in this instance the Ringer's solution took part in their destruction. However, it has been shown that the blood has no marked bactericidal power, but that the lung with the blood still in the capillaries (Experiment 7), the lung perfused with Ringer's solution after the streptococci had been taken up (Table III), and the lung perfused before the streptococci were injected (Experiment 8), all show a marked bactericidal effect; and the conclusion is forced upon one that this power lies in the lung tissue itself.

Incidentally the last experiment mentioned raises a doubt as to the importance of the endothelial opsonin of Manwaring in this process, as the streptococci were taken up and destroyed by a lung which had been previously perfused for over 2 hours in order to free it of blood.

In fact it seems doubtful if humoral protective substances are active here. In one experiment in which a cat that had received streptococci sensitized with immune rabbit serum was tested with another which was given unsensitized bacteria there was little difference

between the two animals; in fact the destruction of the unsensitized cocci was the more marked. Experiments with immune rabbits, which we shall refer to later, indicate that immunization may influence the taking up of the cocci by the tissues, but does not affect the manner or extent of their destruction.

This bactericidal action of the lung tissue which can be demonstrated *in vitro* must doubtless be due to some enzyme or antibody-like substance and we have made repeated attempts to demonstrate some such destructive action in extracts of fresh lung tissue. Portions of lung of freshly killed cats were removed and ground with sand, in fresh cat serum, and separated by brief centrifugation into an opalescent supernatant fluid and a muddy sediment. Both supernatant fluid and sediment were tested by inoculating them with a small amount of streptococcus culture, and plating measured amounts after various periods of incubation. Experiments carried out in this way have failed to show any definite bactericidal effect, though a slight reduction in the number of colonies may appear, as shown in Experiment 9. However, a control tube of the same extract previously heated at 56°C. for 20 minutes showed almost as marked a drop.

Experiment 9.—Lung extract unheated gave 900 colonies immediately; after 1, 2, 4, and 8 hours it gave 800, 600, 2,700 and 3,000 respectively.

Lung extract heated gave 1,000 colonies immediately; after 1, 2, 4, and 8 hours it gave 1,200, 750, 2,000, and 3,000 respectively.

Ground lung tissue gave 150 colonies immediately; after 1, 2, 4, and 8 hours, 450, 750, 1,200, and 3,000 colonies respectively.

It is evident then that streptococci withdrawn from the circulation of normal cats by the lung are promptly killed in the living animal and that pieces of excised lung carry out this same process in the test-tube. The bactericidal power seems to depend, however, on the living conditions of the cells, is much enfeebled by the removal of the tissue from the body, and is not exhibited at all by fresh extracts of the tissue.

With regard to the other organs there seems little doubt that in the living animal the liver destroys many of the organisms taken up, and in some experiments, Table III, for example, this destruction apparently took place *in vitro*.

The disappearance from the blood we believe, for reasons already given, to be due to a taking up and destruction of the bacteria by the viscera and not to any bactericidal power in the blood plasma or cells. The spleen, and in a few experiments the muscle, kidney, and bone marrow, have failed to show bactericidal power *in vitro* and it is impossible to determine from our data whether the disappearance of the streptococci from these organs in the living animal is due to a destruction *in vivo* or to their being carried by the blood to the lung or liver and destroyed there.

Comparison between Susceptible and Resistant Animals.

Our observations on cats have been presented in detail in order to make clear the evidence on which we based our conclusions as to the fate of the injected streptococci. The significance, for the protection of the animal, of this mechanism for taking up and destroying the injected cocci becomes more apparent if we compare the findings in cats which are resistant to the infection with those in rabbits which are susceptible. These we shall present briefly.

The ability of rabbits to free their circulation temporarily of injected streptococci has already been discussed. The subsequent course of the injection is illustrated in Table V.

The first difference between the rabbits and the cats is in the primary distribution of the invading bacteria. In the experiments in Table V about equal amounts were deposited in the lung, liver, and spleen, and in other experiments the number taken by liver and spleen considerably exceeded that taken by the lung (Table VI), whereas in the cats, the lungs as a rule take up several times as many as these other organs. In the rabbits, too, a detectable number was taken up by the muscles and many by the bone marrow.

The findings in the lung of the rabbit killed after 8 hours are similar to the condition in cats killed at the corresponding period, in that the culture of the lung showed a greatly diminished number of streptococci, although they were still found without difficulty in film preparations. At this stage the liver and spleen also appear to have killed most of the organisms, which they have taken up. As in the cats, a feeble bactericidal action by lung tissue could be demonstrated

TABLE V.

Fate of Streptococci in Rabbit Tissues.

Three normal rabbits were injected with a suspension of streptococci, the doses being about 160,000,000 per kilo.

Rabbit 6 was killed in 10 min.

Rabbit 7 was killed after 8 hrs.

Rabbit 8 was found moribund on the 4th day and was killed.

Portions of the organs were weighed, ground with sand, and plated in amounts representing from 0.1 to 0.001 gm. of tissue. The results are recorded as the number of colonies obtained from 0.1 gm. of tissue.

Tissue.	Rabbit 6 (10 min.)		Rabbit 7 (8 hrs.)	Rabbit 8 (4 days)
	Film.	Colonies.	Colonies.	Colonies.
Lung	++	54,000	220	18,000
"		76,000	220	19,000
Spleen	+	34,000	230	5,000
Liver	++	116,000	0	44,000
Kidney		0	0	127,000
Psoas		75	116	98,000
Quadriceps			750	61,000
Heart muscle				80,000
Bone marrow		5,000	0	21,000
Axillary lymph node				59,000
Mesenteric lymph node				190,000
Blood (1 cc.)		1,200	42	6,600

in vitro, whereas streptococci multiply with great rapidity in spleen and muscle tissue removed from the body.

As far as one can determine from the experiments *in vitro* the bactericidal power of lung tissue of the rabbit is distinctly inferior to that of the cat, as is shown in Table IV, where the tissues of the two species were tested after the animals had been given proportional injections. In other cases, however, for example Table VI, rabbit lung tissue showed distinct bactericidal power *in vitro* and the observations on living animals indicate (Table V) that the organisms taken up by a rabbit's lung and liver are largely disposed of. The more striking difference between the susceptible and insusceptible animals is that in the rabbit a considerable number of bacteria lodged in the muscle tissues and sometimes in the kidneys, and that these multi-

plied slowly in the living animal and with remarkable rapidity in the excised tissue.

Just how these organisms are deposited in the muscle tissue of the rabbit we have not yet determined, and in fact a study of this point is difficult because the number primarily taken up is so small that the bacteria are difficult to find in sections. The fact, however, that in this tissue the streptococci are removed from the blood stream and nevertheless proceed to grow, whereas in the lung and liver they are

TABLE VI.

Bactericidal Effect of Rabbit Tissues.

A normal rabbit was injected under ether intravenously with a small dose of streptococci and bled to death in 10 minutes. The lungs were perfused briefly with Ringer's solution and portions placed in test-tubes. The other organs were then perfused and specimens similarly taken. Samples of blood were distributed in 1 cc. amounts and allowed to clot. Pieces of tissue were weighed, ground, and plated at intervals as in previous experiments.

Tissue.	Colonies per 0.1 gm. of tissue tested.				
	Plated immediately.	After 1 hr.'s incubation.	After 2 hrs.' incubation.	After 4 hrs.' incubation.	After 8 hrs.' incubation.
Lung	8,800	1,600	800	1,500	32,000
"	10,000		3,300	3,200	24,000
Spleen	75,000		60,000	360,000	300,000
Liver	24,000		46,000	100,000	300,000
Kidney	140		200	1,500	3,000
Psoas	6		11	110	3,000
Blood	180		100	130	750

removed from the blood stream and killed, forces the conclusion that there must be an essential difference in the way they are held by the muscle tissues to account for their unrestricted multiplication.

Our observations seem to indicate that the susceptibility of the rabbit to the infection is determined not by the inability of the lung and other tissues to kill the streptococci which they take up primarily, but by the fact that a considerable number of the organisms lodge in the muscles and other tissues which lack this bactericidal power and that they multiply almost from the first in these tissues where they are found in enormous numbers at the time of death. The

great number found at the time of death in the lung and liver, organs which were able to free themselves almost entirely of living cocci during the first period of the infection, may well indicate that toward the termination of the infection these resistant organs are overwhelmed with bacteria brought to them from the muscles where they have been growing without hindrance.

Wyssokowitsch[6] considered this possibility. He said:[16] From the experiments so far performed, one cannot determine with certainty whether also in this case (a susceptible animal) the bacteria taken up by certain organs are not destroyed, and whether perhaps only in certain locations the bacteria survive and then multiply. One might suppose that the body is gradually overwhelmed with newly developed bacteria from the least resistant tissue.

Fate of Streptococci in Immune Rabbits.

The next point which we desired to investigate was the effect of active or passive immunization on the rabbit's ability to rid itself of the invading organisms. Our observations on this point are incomplete and unsatisfactory, chiefly on account of the difficulty in producing in rabbits a reliable immunity to the streptococcus. We have treated rabbits for as long as 8 months with intravenous injections of killed streptococci and found that when they were injected with an amount sufficient to kill a normal animal in 2 or 3 days, they usually succumbed. Our investigations are not numerous enough to lay down general rules as to the course of these infections, but it appears that rabbits so immunized live somewhat longer than normal controls, and that after 12 to 24 hours their blood stream becomes free of streptococci. Later they develop a local infection, frequently in the joints, and subsequently succumb to septicemia.

This occurrence of local infection in immunized or possibly sensitized animals opens a field for further study. It would seem to have a direct bearing on the problem of local infections in man. The observations of Wadsworth[17] on pneumonia in rabbits, and of Faber[18] on streptococcus arthritis at once come to mind in this connection.

[16] Wyssokowitsch,[6] p. 40.

[17] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851.

[18] Faber, H. K., *J. Exp. Med.*, 1915, xxii, 615.

An experiment in which the reactions of an immunized and of a normal rabbit to injections of streptococci were compared is recorded in Table VII.

TABLE VII.

Comparison between Normal and Immunized Rabbits.

A normal rabbit and a rabbit which had been treated 2 months with intravenous injections of killed Streptococcus 43 were injected into the left ventricle with 8 cc. per kilo of a suspension of the same streptococcus containing 20,000,000 per cc. They were bled to death under ether 10 minutes after being injected, and the organs treated as in previous experiments.

Tissue.	Colonies per 0.1 gm. of tissue.			
	Immune rabbit.		Normal rabbit.	
	Plated immediately.	After 4 hrs.' incubation.	Plated immediately.	After 4 hrs.' incubation.
Lung	170,000	119,000	61,000	—
"	173,000	128,000	21,000	—
Spleen	4,000	138,000	120,000	1,800,000
Liver	10,000	2,000,000	104,000	6,000,000
Kidney	0	0	0	0
Psoas	31	1,000	1,500	3,000
Quadriceps	130	1,200	2,400	3,000
Blood (1 cc. clotted)	1,000	3,000	—	—

In the brief period of observation the removal of the cocci from the circulation of the immunized rabbit was no more complete, in fact even less so than from that of the normal. Cultures from the carotid 10 minutes after injection showed 35,000 in the immune and 21,000 in the normal animal.

In this instance the lung tissue of the immune rabbit showed a questionable bactericidal power, but at least there was inhibition of growth by the lung as compared with rapid multiplication of the cocci in the other tissues. Similar results have sometimes been obtained with normal rabbit tissues (Table IV). The only marked difference between the immune and the normal animal is in the primary distribution of the cocci. It will be noted that while in the normal rabbit (Tables V and VI) the spleen and liver take up nearly as many or more cocci than the lung, in the immunized rabbit the lung has seized 17 times as many as the liver and 40 times as many as the spleen. In other words, the ratio of distribution of the cocci among

the organs of the immunized rabbit is roughly that found in normal cats.

On account of unavoidable interruption of our work we have been unable to extend further these preliminary observations on the effect of immunization.

CONCLUSIONS.

1. Streptococci injected into the circulation of cats are quickly withdrawn and are found most numerously in the lung, less numerously in the liver and spleen, and in small numbers in the bone marrow, lymph nodes, muscle, and kidney.

2. The streptococci taken up by the lung are killed within 5 to 8 hours, although they remain visible in films for a number of days. In the liver they are killed less rapidly, and in the spleen a few may remain viable for a considerable period.

3. This bactericidal action may be demonstrated in pieces of excised lung but not in lung extracts, and is apparently dependent on the action of the living cell.

4. Streptococci injected into a susceptible animal, the rabbit, are also promptly removed from the circulation, but are distributed in different proportions, the liver and spleen absorbing almost as many as the lung, and the muscles also taking up an appreciable number.

5. As in the cat, the organisms taken up by the lung and liver of the living rabbit are promptly killed. Those which lodge in the muscles, however, multiply rapidly.

6. About the time that the streptococci have begun to develop in the muscles (4 to 8 hours after injection) the number in the blood stream begins to increase.

7. The increase in the blood stream is not due to exhaustion of the mechanism of their removal nor have these organisms acquired a resistance sufficient to maintain them in the blood stream of a normal animal. The septicemia, then, is probably the result of washing out of organisms from the infected tissues.

8. Attempts to immunize rabbits have been unsuccessful, but in certain treated animals the distribution of the organisms among the various organs approached that found in insusceptible animals; *i.e.*, cats.

COMMON COLDS AS A POSSIBLE SOURCE OF CONTAGION FOR LOBAR PNEUMONIA.

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The pneumococcus has been assumed to be one of the causative agents in common colds because of its known pathogenic character and because of its predominance in the mucous discharge in some cases. As far as we know, determination of the types of pneumococcus encountered in these conditions has not been made except in as far as the *Pneumococcus mucosus* type was identified on cultural grounds. For this reason, a series of common colds was examined to determine the types of pneumococci present. This was undertaken not so much from the standpoint of determining the etiological significance of pneumococci in common colds, but rather to determine whether the fixed types occurred. If these were found, common colds would then have to be considered as a source of infection in the spread of lobar pneumonia.

By injecting the nasal secretion or sputum into mice, pneumococci were recovered in 37 out of 65 cases of common colds. By direct plating on blood agar, pneumococci were found in 6 additional cases. Of the total 43 cases, the incidence of type or group of pneumococcus recovered was as follows: Group I, 2 cases; Group II, 2 cases; Group III, 4 cases; Group IV, 35 cases. The cases from which Group II was isolated and one of the Type III cases had been in contact with pneumonia and, with the data available, it is impossible to decide whether they were merely contact carriers or whether the cold was an actual infection by pneumococci due to contact with the pneumonia cases. None of the other cases from which fixed types were isolated had any such contact as far as could be determined.

In determining the type, immune horse sera were employed. Con-

siderable agglutination was encountered with either or both of the horse sera (Type I and Type II) in low dilutions with the strains finally included in Group IV. In the cases where this was marked, further agglutinations were done with rabbit sera which did not give these aberrant reactions, to eliminate the possibility that some of the strains belong to subgroups of the fixed types as described by Avery.[1] Table 1 gives the results of the tests and shows conclusively that the irregular reactions with the horse sera were due to non-specific agglutinins. Two of the Group IV cultures, C 3 and C 5, show some degree of relation in their cross agglutination.

TABLE I.

Type or group.	Case cultures.	Rabbit sera.					
		C 2	C 3	C 5	C 14	Type I	Type II
IV	C 1	—	—	—	—	—	—
IV	C 2	400	—	—	—	—	—
IV	C 3	—	400	100	—	—	—
IV	C 4	—	—	—	—	—	—
IV	C 5	—	100	300	—	—	—
I	C 14	—	—	—	600	800	—
I	C 20	—	—	—	600	800	—
I	Control strain.	—	—	—	600	800	—
II	" "	—	—	—	—	—	400

In three of the four cases yielding Type III, mouse inoculation only was done, so that no data are available on the preponderance of this organism. In the fourth, direct cultures showed the presence of about 75 per cent of this organism. The data available, therefore, except in one instance, are merely an indication that the catarrhal inflammation was due to this type. *Pneumococcus mucosus* has been found by other observers in the secretions of the respiratory tract in conditions other than lobar pneumonia, as well as in otherwise normal individuals. All these individuals, therefore, must be looked upon as potential sources of infection for others or as sources of possible autogenic cases of lobar pneumonia.

The recovery of pneumococci of Type I from two cases of common colds raises the question whether these persons were carriers or

[1] Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

whether the inflammation was due to the pneumococcus. Both cases were markedly prostrated and had a temperature of 102° F. In both cases direct plates showed that the Type I pneumococcus constituted at least 75 per cent of the organisms developing from the secretion, which strongly suggests that the pneumococcus was the etiological agent. If this deduction is correct, we must add some cases of common cold to the already known sources of contagion for lobar pneumonia; namely, the actual case of pneumonia, the convalescent, and the contact carrier (Stillman[2]). One of these cases was reexamined by mouse inoculation whenever a cold recurred over a period of over 2 years. The first reexamination, done a year later, yielded a few Type I pneumococci, most of the organisms in the mouse peritoneal cavity being Pneumococcus Type IV and the bacillus of Friedländer. This shows that the individual was still a carrier of Type I, the small numbers of this organism recovered indicating that it was not of etiological significance in this cold. Subsequent examination for a period of a year, the first a month later, yielded only Pneumococcus Type IV.

SUMMARY.

In two instances of common colds, with no known contact with cases of pneumonia, Pneumococcus Type I was found to be the predominating organism, which strongly suggests that it was the etiological agent in these colds. If this is so, common colds of this type must be looked upon as a possible source of contagion in the development of lobar pneumonia due to the Type I pneumococcus.

[2] Stillman, E. G., *J. Exp. Med.*, 1916, xxiv, 651.

A STUDY OF MITOCHONDRIA IN EXPERIMENTAL POLIOMYELITIS.

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INTRODUCTION.

Although many observations have been made on mitochondria in normal tissues, both adult and embryonic, the study of these structures in pathological material has been relatively limited and the results in some cases have been conflicting. The information regarding this type of cell granule has been summarized by Cowdry,[1] but in view of the fact that several observers have recorded changes in mitochondria, often occurring quite early in certain lesions,[2-13] it seemed possible that some such results might be obtained in the case of poliomyelitis, which would throw light on the pathology of that condition.

Spinal ganglia were employed for the study, because they show typical lesions in monkeys dying of experimental poliomyelitis, and because, of the structures showing these lesions, they could be most

[1] Cowdry, E. V., *Am. J. Anat.*, 1916, xix, 423.

[2] Barrett, J. O., *Quart. J. Micr. Sc.*, 1913, lviii, 214.

[3] Beckton, H., *Arch. Middlesex Hosp.*, 1909, xv, 182.

[4] Beckton, H., and Russ, S., *Arch. Middlesex Hosp.*, 1911, xxiii, 59.

[5] Bensley, R. R., *Tr. Chicago Path. Soc.*, 1909–12, viii, 78.

[6] Ciaccio, C., and Scaglione, S., *Beitr. path. Anat. u. allg. Path.*, 1913, lv, 131.

[7] Goetsch, E., *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 29.

[8] Homans, J., *J. Med. Research*, 1915–16, xxxiii, 1.

[9] Regaud, C., and Favre, M., *Compt. rend. Soc. biol.*, 1911, lxviii, 658.

[10] Regaud and Favre, *Compt. rend. Soc. biol.*, 1912, lxix, 328.

[11] Romes, B., *Anat. Anz.*, 1913–14, xlv, 1.

[12] Scott, W. J. M., *Am. J. Anat.*, 1916, xx, 237.

[13] Strongman, B. T., *Anat. Rec.*, 1917, xii, 167.

successfully obtained and fixed. Cowdry[14] has given a full account of the mitochondria occurring in normal ganglion cells of vertebrates.

EXPERIMENTAL.

In a few instances injection fixation was employed. Here the method described by Cowdry[1] was used with the following variations. The formalin bichromate mixture was more successful when used in half rather than in full strength. 2 to 3 feet of gravity pressure of injection fluid were found to give less edema and distortion of the tissues than the higher pressure (4 to 6 feet), so that while the saline infusion was given at the higher pressure for the first 5 to 10 minutes to insure complete washing out of clots, the lower pressure was maintained throughout the remainder of the period. The femoral vein of one side was cut, rather than the vena cava, as the latter gave too rapid an outflow of fluid. The cardiac, mesenteric, and opposite iliac arteries were clamped. The injection of fixative was continued for 1 hour and the best results were obtained when the animal was kept lying on the board back down, for $\frac{1}{2}$ hour longer before autopsy.[15] Injection was less successful in poliomyelitic monkeys and in the lumbar region of operated rabbits than in normal animals, possibly owing to vascular lesions in the former.

The ganglia, however, were quite as well fixed by simple immersion in fixing fluid. The animals were usually chloroformed and the ganglia taken at once. In a few cases the animals died and were autopsied within 2 hours of death. The specimens were placed in formalin bichromate mixture (3 per cent potassium bichromate 4 parts, neutral formalin 1 part, and water 5 parts) for 4 days and then transferred to half strength ($1\frac{1}{2}$ per cent) potassium bichromate for 5 days. This procedure was found to give better results than the solution ordinarily used. Full strength solutions and the acetic-osmic-bichromate mixture[16] (2.5 per cent bichromate 8 cc., 2 per cent osmic acid 2 cc., and glacial acetic acid 1 drop) in full strength or diluted to half strength were apt to give good fixation only in the

[14] Cowdry, *Am. J. Anat.*, 1914–15, xvii, 1.

[15] Schirokogoroff, J. J., *Anat. Anz.*, 1913, xliii, 522.

[16] Bensley, *Am. J. Anat.*, 1911–12, xii, 297.

case of superficial cells. The osmic acid mixture also darkened the whole section to such an extent that it dimmed the contrast between mitochondria and small Nissl bodies.

At first the use of chloroform in embedding was tried, but in many cases the mitochondria disappeared under this treatment. In one case the tissue was first embedded by the xylol method and sections were cut, then reembedded by the chloroform method.[17] The former sections showed mitochondria, but none was present in the latter. This may have been due to rehandling, however. Embedding was done as described by Cowdry,[14] except that absolute alcohol-xylol for 1 hour, xylol 1 hour, paraffin 3 hours, was found to be sufficient and less liable to destroy the mitochondria. Both the acid fuchsin-methyl green, and the iron-alum-hematoxylin (Regaud and Favre[9]) methods were used for staining.

Rabbits.—In order to compare cell changes in another form of paralysis a series of rabbits was used, in some of which ischemic paralysis of the hind legs was produced by the Stenson operation (Fredericq,[18] Ehrlich and Brieger[19]). The animals were etherized. An area about 3 inches wide, extending from ensiform process to symphysis pubis, was shaved and cleaned with alcohol. Aseptic technique was employed. An incision was made in the midline, and the intestines were covered with cloths wet with warm saline. The abdominal aorta was exposed and a soft bulldog clamp placed on it about $\frac{1}{2}$ inch below the renal arteries, the effectiveness of the clamp being tested by palpation of the vessel below it. The intestines were replaced and the animal was kept under light ether anesthesia, the clamp being left in place for $\frac{1}{2}$ or $\frac{3}{4}$ hour. The longer period was found to give certain results while the former failed in some cases to give paralysis. Care was taken to keep the animal warm during this period. At the end of the period the clamp was removed, the abdominal wall sewed with silk, and the animal allowed to recover.

In almost every instance, flaccid paralysis of the hind legs was evident as soon as the animal recovered from the ether. In two rabbits

[17] Mallory, F. B., and Wright, J. H., Pathological technique, Philadelphia and London, 6th edition, 1915, 284.

[18] Fredericq, L., *Arch. biol.*, 1890, x, 131.

[19] Ehrlich and Brieger, *Z. klin. Med.*, 1884, vii, Supplement, 155.

in which the shorter compressions were used, the hind legs were spastic, with convulsive twitchings, which gradually disappeared, the animal recovering the full use of the legs.

The animals were chloroformed at various periods after the ligation and fixed by the injection method. The lumbar region of the cord did not always take the fixative so well as did the cervical region, or the lumbar region of normal animals, and in those animals killed 12 or more hours after the clamping, the cords remained extremely soft, and were uncolored by the chromate and very difficult to handle.

Material.—A series of ganglia was obtained from fourteen monkeys with experimental poliomyelitis.[20] Six were either in the preparalytic stage, without definite lesions in the ganglia, though having shown such symptoms as irritability, etc., or if they showed paralysis, the particular ganglia used failed to show typical lesions. The remaining ten, taken from the 1st to the 7th day after the onset of paralysis of some muscle group had been noted, all showed typical lesions of the cord and ganglia, including those used for mitochondria, as shown by examination in gross or of sections stained by hematoxylin and eosin.

Five monkeys were used as controls. Two of these showed lesions of tuberculosis at autopsy. One was an apparently normal monkey which died while being etherized, and the viscera showed no gross abnormalities. The other two monkeys had received poliomyelitis virus intranasally but showed no symptoms either during life or post mortem.

Results.—The ganglia from the five control monkeys and those from the six poliomyelitic monkeys presenting no lesions in the ganglia used showed mitochondria similar to those described by Cowdry in the normal animal. Great variations were found in the number of mitochondria and the intensity with which they took the stain, and several showed many cells in the chromatophilic state.[21] In one, considerable postmortem degeneration had occurred.[22] One showed a large amount of typical lipoid. All, however, were cells similar to those described by Cowdry as normal cells. This was true also

[20] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

[21] Cowdry. Contributions to embryology, *Carnegie Institution of Washington, Publication No. 224*, 1916, Contribution No. xi.

[22] Ciaccio, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 721.

of the ganglion cells from the normal rabbits and those from the cervical region of the operated rabbits. In these rabbit sections, no chromatophil cells were found.

In the tissues from the poliomyelitic animals also, many cells were normal in appearance. In many, moreover, the mitochondria appeared to be even more clearly shown than in normal cells. This appearance may have been due to disappearance of Nissl substance, which was reduced in these cells. In normal cells it was often hard to differentiate between mitochondria and small Nissl bodies as the granules were of nearly the same size and in some preparations tended to take the fuchsin stain. Many cells contained much particulate lipoid and many were in the chromatophilic state, but in these particulars they did not seem to exceed normal limits.

In the cells which showed marked neurophagocytosis, mitochondria-like threads could often be seen, even though only a small remnant of protoplasm remained. They appeared as minute reddish threads or dots, or larger masses, even to fairly large rods, lying in the usual bluish background and in spaces between the invading cells. They did not have the globular shape characteristic of lipoid, and they seemed to have some of the chemical reactions of the mitochondria, for they were not found in slides in which the mitochondria had been lost through poor fixation. In cells in which the destruction had been less complete, typical mitochondria were found to persist in an apparently normal ratio to cell substance.

A similar, though less marked persistence of mitochondria was also noted in the lumbar ganglia of the rabbits. 1 hour after the production of the anemia, a few darkly staining cells were found, showing a few reddish threads against a dark purplish background. At 7 hours almost all the cells had this chromatophilic tendency, a few still showing the reddish threads, but in the majority there was only a shrunken, irregularly stained protoplasm. At 12 hours, the position of a few remaining cells was indicated by dark, indefinite spots.

DISCUSSION.

If these red-staining threads occurring in the invaded cells in poliomyelitis are mitochondria, the mitochondria in this condition

at least outlast any other cell structure now recognized. This is remarkable in view of their usual tendency to disappear under slight changes, such as acidity or temperature. Since the stain is not absolutely specific, and we lack means at the present time of differentiating mitochondria with certainty from other lipoidal structures, it may be that these threads, or rods, are merely part of a coagulum of some different nature. Yet, since typical mitochondria persist after the disappearance of typical Nissl substance, and various gradations can be traced up to the stage of almost total replacement of the original ganglion cell, it would seem safe to call these mitochondria under the present use of the term.

It would be desirable to have some means of quantitative estimation of the mitochondria for these purposes, but the one so far described (Thurlow[23]) does not prove applicable to the rod-like forms.

CONCLUSION.

Typical mitochondria can be found in the spinal ganglion cells of monkeys with experimental poliomyelitis, even when typical Nissl substance has disappeared, and mitochondria-like structures are found in the remaining protoplasm in the latest stage of neurophagocytosis.

[23] Thurlow, M., Contributions to embryology, *Carnegie Institution of Washton, Publication No. 226*, 1917, Contribution No. xvi.

THE LOCALIZATION OF STREPTOCOCCUS VIRIDANS.

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Plates 1 and 2.

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The theory of the power of selective action on the part of bacteria, with regard to the tissues in which they may localize, is by no means a new one. We have known for many years that the meningococcus has a predilection for the meninges, *Bacillus diphtheriæ* for the mucous membrane of the throat, *Bacillus typhosus* for lymphoid tissue, the pneumococcus for the lungs, and *Bacillus pertussis* for the tracheal mucous membrane. But that different strains of the same organism should possess distinct localizing power and should vary consistently in their selective action is not generally accepted.

Since Rosenow (1) put forward this theory in 1915, there has been a great deal of discussion on the subject and widely divergent views have been expressed. This observer claims that streptococci, in particular, possess the power of elective localization and has attempted to substantiate his statements by animal experiments on a large scale. He maintains that (1) streptococci isolated from gastric ulcer, appendicitis, cholecystitis, endocarditis, myositis, and neuritis when injected into animals tend to produce lesions similar both in location and in kind to those from which they were originally obtained; (2) this power of selective action is lost sooner or later upon subculture of the organism on artificial media. It was with a view to determine just how far these observations could be confirmed that the present work was undertaken.

In 1914 Rosenow published his newer methods of investigating the bacteriology of various diseases (2), giving his technique for obtaining cultures from diseased tissues and from foci of infection. In 1915 his paper (1) appeared demonstrating the streptococcus as an etiologic factor in nine diseases, showing that the organism could be obtained in pure culture from the lesions and from foci of infection in patients, that when these organisms were injected into animals they localized and produced a higher percentage of lesions in the organs corresponding to those

affected in the patients than in those not so affected, and that this power is lost in a short time when the organism is grown on artificial media. The site of localization is shown to be changed by animal passages and this he associates with a corresponding increase of virulence. Since then he and his colleagues have claimed an elective affinity of streptococci for the ovaries (3) in ovaritis, for the ganglia and posterior roots in herpes zoster (4), for the nerve trunk, muscles, and periosteum in facial neuralgia due to an alveolar abscess (5), for the gall bladder in cholecystitis (6, 7), and in an extensive review of gastric and duodenal ulcer (8) he has concluded that streptococci from gastric ulcers and from foci of infection in these cases have a selective affinity for the gastric mucosa, while streptococci from duodenal ulcers produce in rabbits ulcers in the duodenum more frequently than in the stomach.

Oftedal (9) found evidence of elective localization of streptococci isolated from the sputum of patients with bronchial asthma, in the bronchi and lungs of rabbits. Irons, Brown, and Nadler (10) stated that a streptococcus isolated from the tear sac of a patient suffering from iridocyclitis produced iridocyclitis in rabbits. On the other hand, Henrici (11) found that rheumatic lesions in rabbits are produced in equal proportions by both hemolytic and non-hemolytic streptococci, and he does not recognize any class of streptococcus as specific for rheumatic fever. He also found after using fifty-three strains that carbohydrate fermentation tests are of no significance from the standpoint of tissue localization. Moody (12) found that streptococci from chronic alveolar abscesses in patients with arthritis produce in rabbits most lesions in the joints, and that streptococci from the same sources in patients who have lesions other than arthritis and in patients who have no other ailment, produce most lesions in the stomach. Celler and Thalhimer (13) state that intravenous inoculation of streptococci, isolated from cases of gastric ulcer, yielded gastric lesions in 13.3 per cent.

EXPERIMENTAL.

In the work here reported strains of *Streptococcus viridans* only were used. The plan followed was to use each culture of streptococcus immediately upon isolation. The reason for this, of course, is that it is claimed (1) that strains of streptococci lose their special localizing power if subcultured. In some instances the strains were used as isolated and again after several subcultures had been made. It frequently happens, however, that it is impossible for some reason to try out a strain at once, but in these cases the fact is indicated in the reports. The number of rabbits used for the first inoculation was usually three, but this also was not strictly adhered to because of circumstances which we could not control. The organisms were obtained from many different sources and from patients suffering

from a diversity of ailments, in an endeavor to make the series as comprehensive as possible.

Technique.

The strains of streptococci employed in these experiments were obtained by recognized methods of bacteriological procedure. In cases of endocarditis the technique of blood culture was that described in 1916 (14), while in the instances in which the affected valves were cultured, the tissues were treated after the manner described by Rosenow (2). This was also carried out in culturing appendices, gall bladders, and other tissues. Cultures from the tonsils were obtained by expressing the cheesy material and other debris from the tonsillar crypts after swabbing the surface with sterile gauze. Extirpated tonsils were washed in sterile saline solution, then carefully opened, and material from the depths was cultured.

Abscesses at the apices of teeth were cultured in the dentist's office or at the dental clinic at the hospital. After swabbing the area of operation with 2 per cent iodine, the tooth was extracted with great care to prevent contamination, and cultures were taken from the apex. Sometimes the tooth was broken open and cultures of *Streptococcus viridans* were obtained from the pulp. The technique in connection with cases of pyorrhea alveolaris was simple and effective. Preparation of the area was carried out as described and a fine capillary pipette with bulb attached inserted between the tooth and the gum. The point can usually be thrust to quite a depth and the bulb is never released until the point is at the bottom of the pocket. In these cases *Streptococcus viridans* in pure culture results in nearly every instance.

Rabbits about 8 to 12 weeks old were used, and were inoculated intravenously, in the marginal ear vein. The preparation of the cultures for inoculation is described in a previous communication (14). The doses were large but not sufficient to cause immediate or very early death, except in a few instances. The average time elapsing between the date of inoculation and that of postmortem examination was 3 to 4 days. Postmortem examination was performed upon the animals immediately following their death. Many were chloroformed and examined 48 and 72 hours after inoculation. Sections of

all the important organs were cut and suitably stained and these aided materially in the search for lesions.

It is convenient to divide the results into four parts according to the origin of the organism studied.

TABLE I.

Organisms Obtained from the Appendix.

Organ.	Lesions with direct cultures; 4 strains and 14 animals.		Lesions with 1st, 2nd, and 3rd subcultures; 7 strains and 12 animals.		Lesions with older subcultures; 2 strains and 6 animals.		Lesions with animal passage strains; 2 strains and 7 animals.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
Brain	—		2	17	—		—	
Meninges	—		—		—		—	
Spinal cord	—		—		1	17	1	14
Myocardium	3	21	6	50	1	17	4	57
Endocardium	2	14	2	17	1	17	3	43
Pericardium	—		1	8	—		—	
Lung	2	14	2	17	—		—	
Pleura	—		1	8	—		—	
Diaphragm	1	7	1	8	—		—	
Thymus	—		1	8	—		—	
Stomach	4	29	1	8	—		—	
Duodenum	3	21	—		1	17	—	
Gall bladder	—		—		—		—	
Liver	1	7	2	17	1	17	1	14
Intestine	—		—		—		—	
Appendix	2	14	2	17	—		—	
Kidney	—		2	17	—		—	
Spleen	—		—		—		—	
Muscles	2	14	—		—		—	
Joints	5	36	2	17	—		4	57
Periosteum	—		—		—		—	
Skin	—		1	8	—		—	
Peritoneum	—		1	8	—		—	
Aorta	—		—		—		—	

Organisms Isolated from Infected Appendices.—These organisms were obtained from the serosa or the wall of the appendix, the adjacent lymph glands, and two or three strains from the lumen or from pus in a localized appendical abscess were used after it had been shown that they did not ferment mannite.

Organisms Isolated from the Gall Bladder.—We laid little emphasis on whether the organism was obtained from the contents, the mucosa, or from the wall, believing that when a streptococcus is obtained in pure culture from any of these sites and no other organism is found, that it is the cause of the lesion and is useful for experimental work.

TABLE II.

Organisms Obtained from the Gall Bladder.

Organ.	Lesions with direct cultures; 4 strains and 13 animals.		Lesions with animal passage strains; 2 strains and 3 animals.	
	No.	Per cent.	No.	Per cent.
Brain	2	15	—	
Meninges	—		—	
Spinal cord	—		—	
Myocardium	5	38	—	
Endocardium	4	31	—	
Pericardium	—		—	
Lung	2	15	—	
Pleura	—		—	
Diaphragm	—		—	
Thymus	—		—	
Stomach	4	31	—	
Duodenum	—		—	
Gall bladder	1	8	—	
Liver	1	8	—	
Intestine	—		—	
Appendix	—		—	
Kidney	1	8	—	
Spleen	—		—	
Muscles	—		1	33
Joints	6	46	1	33
Periosteum	—		—	
Skin	—		—	
Peritoneum	—		—	
Aorta	—		—	

Organisms Isolated from the Tonsils.—In these cases the tonsils selected were those clinically known to be diseased and regarded as a probable focus of infection in patients suffering from various ailments. We avoided mouth contamination by culturing after removal and used only pure cultures of streptococci obtained from the deep tonsillar tissue.

Organisms from Blind Apical Abscesses and Pyorrheal Pockets.—As in the case of infected tonsils, the cases selected were believed to have foci of infection in these areas and only pure cultures of streptococci were used.

TABLE III.

Organisms Obtained from the Tonsil.

Organ.	Lesions with direct cultures; 3 strains and 9 animals.		Lesions with 1st, 2nd, and 3rd subcultures; 3 strains and 2 animals.		Lesions with animal passage strains; 2 strains and 3 animals.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.
Brain	1	11	—		—	
Meninges	—		—		—	
Spinal cord	—		—		—	
Myocardium	2	22	1	50	—	
Endocardium	3	33	1	50	—	
Pericardium	1	11	—		—	
Lung	1	11	—		1	33
Pleura	—		—		—	
Diaphragm	—		—		—	
Thymus	2	22	—		—	
Stomach	3	33	—		—	
Duodenum	—		—		—	
Gall bladder	—		—		—	
Liver	—		—		2	67
Intestine	—		—		—	
Appendix	2	22	—		—	
Kidney	—		—		—	
Spleen	—		—		—	
Muscles	1	11	—		—	
Joints	1	11	—		—	
Periosteum	—		—		—	
Skin	—		—		—	
Peritoneum	—		—		—	
Aorta	—		—		—	

Allowance was also made for the age of the strain used, and again four classes were made: (1) direct culture; (2) organisms of first, second, or third subcultures; (3) organisms older than the third subculture; (4) organisms after animal passage.

Appendix (Table I).—Strains used 12. Animals used 39.

It will be noted that the largest percentage of lesions is found in the myocardium. Next come joints and endocardium. No lesions are found in the gall bladder or the intestine. When the strain is young the lesions in the appendix are as frequent as in the endocardium and more frequent in the stomach than in the appendix. Later

TABLE IV.

Organisms Obtained from Tooth Abscesses and Pyorrhea.

Organ.	Lesions with direct cultures; 6 strains and 13 animals.		Lesions with 1st, 2nd, and 3rd subcultures; 3 strains and 7 animals.	
	No.	Per cent.	No.	Per cent.
Brain	—		1	14
Meninges	—		—	
Spinal cord	—		—	
Myocardium	6	46	5	71
Endocardium	7	54	4	57
Pericardium	—		—	
Lung	—		1	14
Pleura	—		—	
Diaphragm	1	8	1	14
Thymus	2	15	—	
Stomach	2	15	—	
Duodenum	—		—	
Gall bladder	—		—	
Liver	4	31	3	43
Intestine	2	15	—	
Appendix	5	38	1	14
Kidney	5	38	2	29
Spleen	—		—	
Muscles	—		—	
Joints	2	15	1	14
Periosteum	1	8	—	
Skin	—		—	
Peritoneum	—		—	
Aorta	—		—	

none are found in the stomach or appendix, while there are more in the endocardium.

Gall Bladder (*Table II*).—Strains used 4. Animals used 16.

Here joints are first, followed by myocardium and by endocardium and stomach, with an equal percentage of lesions in each of the

two latter. After animal passage no lesions are produced in the gall bladder, but are observed in the muscles and joints.

Tonsils (*Table III*).—Strains used 5. Animals used 14.

Although the lesions in the heart are most frequent, there is a wider variation of organs showing a high percentage of lesions than is found in strains from gall bladder or appendix.

Tooth Abscesses and Pyorrhea (*Table IV*).—Strains used 9. Animals used 20.

Again although the largest percentage of lesions is in the heart, there is a wide variation of organs attacked.

Taking our results *in toto*, regardless of the origin or age of the strain, we find most lesions in the myocardium, followed closely by the endocardium and joints.

DISCUSSION.

The lesions which we observed were both gross and microscopic; ulceration of the stomach, vegetations on the endocardium, turbid fluid in the joints, and hemorrhage on the mucous and serous surfaces being typical of the former, while the latter may conveniently be discussed under three types.

Hemorrhage.—Besides those observed in the gross and verified by microscopic examination, we frequently found areas of hemorrhage, not visible to the naked eye, which were quite extensive microscopically and which showed no leukocytic reaction around the margin of the hemorrhage. The agency by which this lesion is brought about is in all probability a minute bacterial embolus which lodges in the vessel, invading and weakening the wall and thus leading to rupture. Although there may be no reaction around the margin of the hemorrhage we have seen it around these vessels.

Bacterial Emboli.—Frequently in examining sections of tissue involved, one encounters a picture with the following characteristics: Small masses of bacteria, bacterial emboli, are seen, which may be distinctly in a small vessel or which may have no apparent relation to one. Sometimes the embolus forms the nucleus of a microscopic abscess and again there is no apparent cellular reaction. It is possible that these are different pictures of one process and that distal to the vessel blocked by an embolus and surrounded by leukocytes

there may be an area of hemorrhage. Serial sections would be necessary to decide this question (Figs. 1 and 2).

Areas of Leukocytic Infiltration.—This is perhaps the most common type of lesion and occurs in all organs (Fig. 3). The cells are mostly lymphocytes with usually a few polymorphonuclears scattered throughout. Such areas occur around blood vessels which are apparently normal, around masses of bacteria, and sometimes without apparent relation to either but in relation to an area of necrosis or degeneration of parenchymal cells (Fig. 4).

Although our results on the whole do not favor the idea of elective localization, a few individual strains produced some evidence which is worth recording.

Strain A 16.153 was a *Streptococcus viridans* obtained from an appendix abscess. The second subculture was injected into Rabbit 1, which soon afterwards commenced to be restless and irritable. In 30 hours the rabbit had convulsions which were repeated at intervals for the next 10 hours, when it died. The autopsy showed hemorrhages in the cortex of the brain.

Rabbit 2 had a similar injection, showed the same early restlessness, had convulsions in 30 hours, died in 36 hours, and had fine hemorrhages in the cortex of the brain.

Rabbit 3 was injected with the streptococcus recovered at autopsy from the heart's blood of Rabbit 2. At precisely the same hour as before the rabbit had convulsions followed by paraplegia. The patellar reflexes were increased. In 36 hours it was chloroformed and autopsy revealed a diffuse hemorrhage covering two segments in the lower dorsal region of the cord.

Rabbit 4 was injected with the original strain after 3 weeks of laboratory subculturing. On the 7th day it was found to have paraplegia and was chloroformed. The autopsy showed a diffuse hemorrhage with softening in the sacral enlargement of the cord.

After further subculture for 10 days the strain produced lesions in the heart and joints only, and, after five animal passages, failed to regain its virulence for the nervous system. Why a streptococcus causing appendicitis in a patient should in this one instance so persistently attack the nervous system we are unable to explain.

Strain D. C. 3, a *Streptococcus viridans* from an alveolar abscess, produced focal glomerular nephritis in each of three rabbits. Many of the glomeruli contained masses of bacteria with surrounding degeneration and leukocyte infiltration. Other similar instances occur in our records.

Such evidence as this seems to us to support the idea that there may be a tendency for a strain of streptococcus to localize in a certain tissue peculiar for that strain but without relation to its origin.

CONCLUSIONS.

The results of our experiments do not substantiate in full the theory of the power of selective action of *Streptococcus viridans*.

A few strains showed a remarkable constancy in location and type of lesion, but these strains were greatly in the minority.

The location of the lesions in animals seemed to bear no relation to the origin of the organism or to the lesions produced by it in the patient from which the strain was obtained.

Streptococcus viridans, regardless of site of origin in the patient, produced most lesions in the heart and joints.

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EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Heart muscle; vessels plugged with bacteria. Perivascular infiltration.
FIG. 2. Glomerulus nearly filled with bacterial growth.

PLATE 2.

FIG. 3. Periportal round celled infiltration.
FIG. 4. Cortex of brain. Note the perivascular round celled infiltration and degeneration, also meningeal involvement.

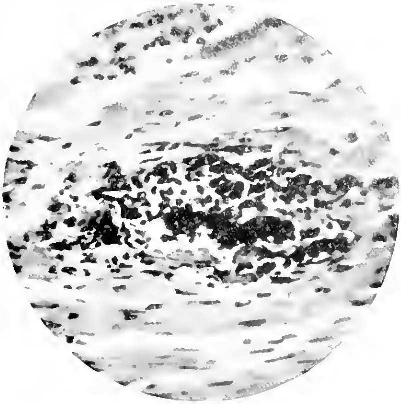

FIG. 1.

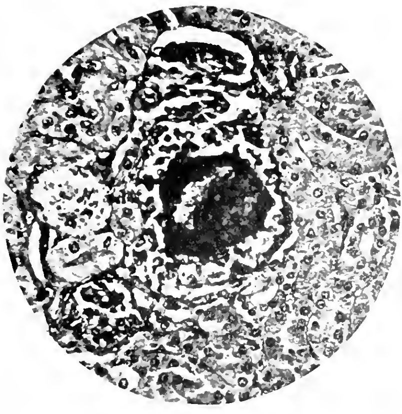

FIG. 2.

(Detweiler and Maitland: Localization of *Streptococcus viridans*.)

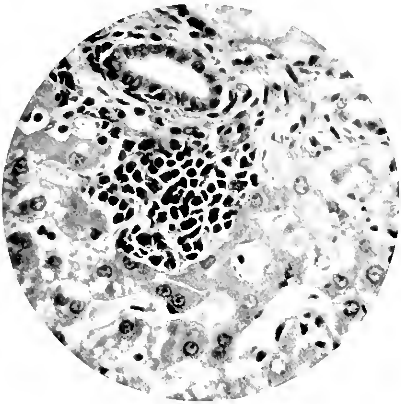

Fig. 3.

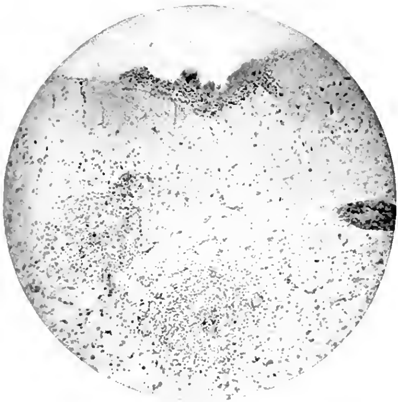

Fig. 4.

(Detweiler and Maitland: Localization of *Streptococcus viridans*.)

MORPHINE HYPERGLYCEMIA IN DOGS WITH EXPERIMENTAL PANCREATIC DEFICIENCY.

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(Received for publication, October 11, 1917.)

In a recent investigation[1] we found that after largely abolishing the function of the living pancreas in dogs, without resecting this gland, there was usually no marked hyperglycemia or glycosuria. Occasionally, however, there occurred rises in the glycemia of some of these animals which were greater than those generally observed in the normal dogs. As these animals had less than 5 per cent of unkilled pancreatic tissue remaining and were entirely without any external pancreatic secretion, we looked upon this occasional hyperglycemia as a sign of weakness in the carbohydrate metabolism. The idea then suggested itself that any factor causing a hyperglycemia would probably call forth a greater response in animals with a pancreatic deficiency than in normal individuals. This conception was readily put to an experimental test, and we may state that our expectations were fulfilled.

Method.

Dogs only were employed, and a pancreatic deficiency was produced in four ways. In the first method the pancreas was largely coagulated by injecting alcohol-acetic acid into the main excretory duct, the accessory duct being clamped or ligated. In successful experiments less than 5 per cent of the pancreas remains uncoagulated. In the second group of dogs, the entire pancreas was resected, but the uncinate process with its blood supply intact was transplanted to the subcutaneous tissue in a one stage operation. It should be noted that both classes of dogs were deprived of their pancreatic digestive juices. In another type the pancreas was largely resected, but approximately one-sixth of the gland about the excretory ducts was allowed to remain. Such an animal

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[1] Auer, J., and Kleiner, I. S., Preliminary note, *Proc. Soc. Exp. Biol. and Med.*, 1917, xiv, 151.

therefore is not deprived entirely of its pancreatic digestive juices. In the fourth class the uncinate process and tail of the pancreas were merely ligated, leaving about one-third of the structure in connection with the excretory ducts. All operations were performed under full ether anesthesia.

The method for causing hyperglycemia gave some trouble, for we desired a procedure which would not subject the animals to an undue strain, as our first study on the effects of coagulation of the pancreas *in situ* was not completed and we were using the same animals. The method therefore should be as harmless as possible, and in addition should not entail the use of glucose or other sugars in any way. These demands were satisfied by injecting morphine subcutaneously.

The dose of morphine sulfate was small, usually 2 mg. per kilo of body weight, and the site of injection was the subcutaneous tissue of the chest.

All the dogs with an experimental pancreatic deficiency had been operated 1 to 4 months before the morphine test.

As controls we used apparently normal dogs, or dogs which had been fasted for either 8 or 22 days.

All the dogs, except those fasting, were fed once daily a diet composed of about 100 gm. of cooked meat scraps, and 400 to 500 gm. of bread-meat broth mixed with ground bone.

The samples of blood were invariably drawn from an external jugular vein into a syringe containing a small amount of sodium oxalate. In general, four samples of 2.5 cc. of blood were drawn from each dog: a normal sample, and then three more at hourly intervals calculated from the time of the morphine injection.

After obtaining the blood sample, the glycemia was determined by the Lewis-Benedict method as modified by Myers and Bailey.[2]

RESULTS.

Four dogs, all females, were used in the first series of experiments. Two of them, Dogs AK5 and AK32, were theoretically in the prediabetic stage, as at least 95 per cent of the pancreas had been destroyed in each by coagulation 4 months and $1\frac{1}{2}$ months ago respectively. Neither showed a glycosuria. The glycemia in Dog AK5 had ranged between 0.10 and 0.15 per cent; in Dog AK32 between 0.09 and 0.19 per cent, the latter figure being reached only twice before the morphine experiment was made. The two remaining dogs were normal animals and served as controls. All four dogs received 2 mg. of morphine sulfate per kilo subcutaneously in the chest. They had been fed 4 hours before.

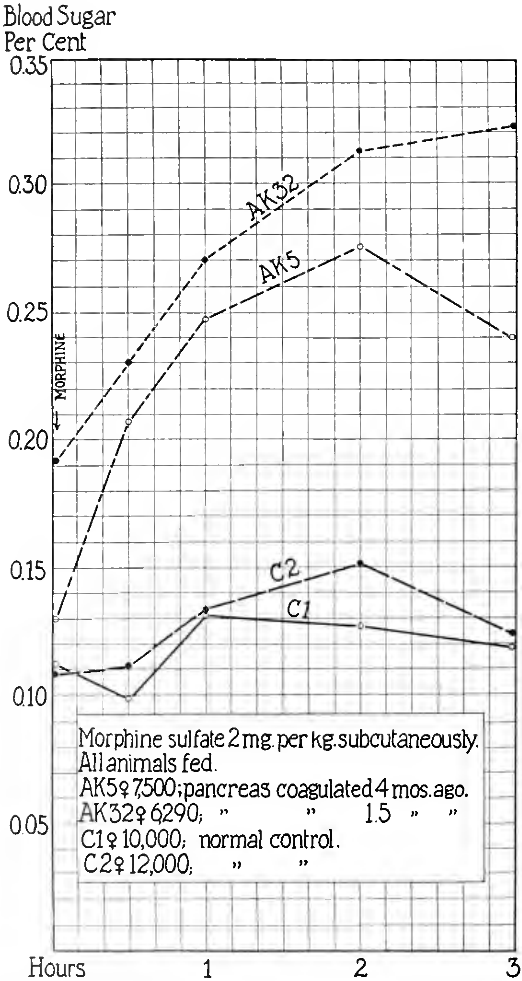
[2] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.

The changes in the glycemia are striking and are brought out by the curves of Text-fig. 1. It will be seen that the two controls showed but a slight increase in the glycemia during the 3 hours following the morphine injection, the maximum level reached being 0.15 per cent, an increase of only 0.04 per cent over the normal.

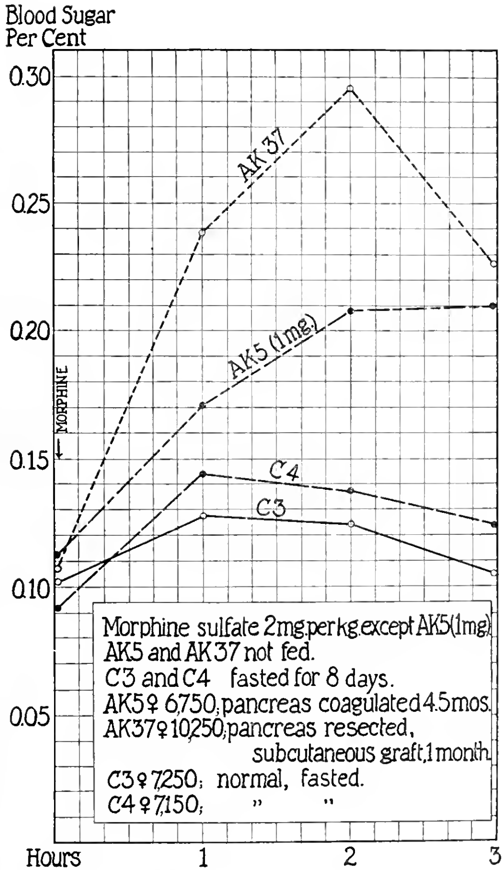
In the dogs with pancreatic deficiency, however, there was a tremendous rise in the blood sugar after the morphine. With Dog AK5 the blood sugar rose from 0.13 to 0.28 per cent after 2 hours; in Dog AK32, from 0.19 to 0.32 per cent in the same length of time. In these animals, therefore, the same dose of morphine caused a rise of 0.15 and 0.13 per cent respectively in the blood sugar, increases which are three to four times greater than those observed in the controls.

In the second series of experiments two dogs with pancreatic deficiency and two controls were employed. One of the prediabetic dogs was again Dog AK5; the second one was Dog AK37 whose pancreas with exception of the uncinate process had been resected 1 month previously, the uncinate portion with intact blood supply being transplanted to the abdominal subcutaneous tissue. The latter dog had no glycosuria beyond an occasional faint trace, and the blood sugar had ranged between 0.09 and 0.15 per cent. The controls were normal animals which had been fasted for 8 days. The prediabetic dogs, Nos. AK5 and AK37, were not fed on the day of the morphine experiment. The two controls and the dog with the subcutaneous pancreatic graft (Dog AK37) received 2 mg. of morphine sulfate per kilo subcutaneously; Dog AK5, however, was only given 1 mg. per kilo.

The results are clearly shown in Text-fig. 2. Here again we observe a striking quantitative difference in the glycemia of the two groups. The control dogs, starting from the 0.09 to 0.10 per cent level, show merely a rise of 0.05 per cent within 2 hours after the morphine. The prediabetic dogs, on the other hand, though beginning practically with the same glycemia as the controls, develop within 2 hours after the morphine a hyperglycemia of 0.21 per cent in Dog AK5 and 0.30 per cent in Dog AK37, levels which represent rises of 0.10 and 0.19 per cent in the blood sugar respectively. It must also be remembered that one prediabetic dog, No. AK5, received only half the amount of morphine per kilo which was given to the controls.



TEXT-FIG. 1. Influence of coagulation of the pancreas on morphine glycemia.



TEXT-FIG. 2. Influence of two types of pancreatic deficiency on morphine glycemia.

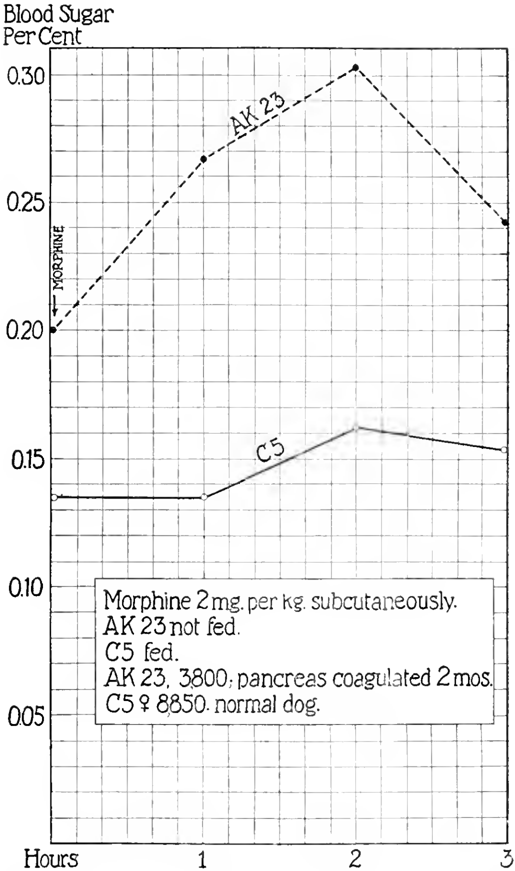
In a third group the effect of 2 mg. of morphine per kilo showed the same general difference described before, and is graphically shown in Text-fig. 3. The pancreatic deficiency in Dog AK23 was caused 2 months previously by coagulating most of the pancreas *in situ* with alcohol-acetic acid. The glycemia of this dog since operation had ranged between 0.120 and 0.20 per cent, the latter figure being reached only occasionally. There was no glycosuria except an occasional faint trace. This dog was not fed on the day of the experiment, but through an oversight the control, Dog C5, was fed 2 hours before. The latter dog vomited a large amount of food within a few minutes after the morphine was given.

From the curves of Text-fig. 3 it will be seen that the control's blood sugar rose from 0.136 to 0.161 per cent within 2 hours after the morphine administration, an increase of 0.025 per cent. The other dog, however, with deficient pancreas showed a glycemia which rose from 0.20 to 0.306 per cent within 2 hours after the morphine, a rise of 0.10 per cent, or four times more than the control.

In a fourth series we studied the effect of morphine when administered to two fasting dogs with the pancreas intact. The fasting period had lasted 22 days, the animals having free access to water. Both dogs weighed originally 8,750 gm. Dog C3 lost 2,900 gm., and Dog C4, 2,750 gm. during the fasting period. The control, Dog C6, was a normal dog weighing 7,500 gm.; it had not been fed on the day of the morphine test. The amount of morphine was 2 mg. per kilo, given subcutaneously as usual.

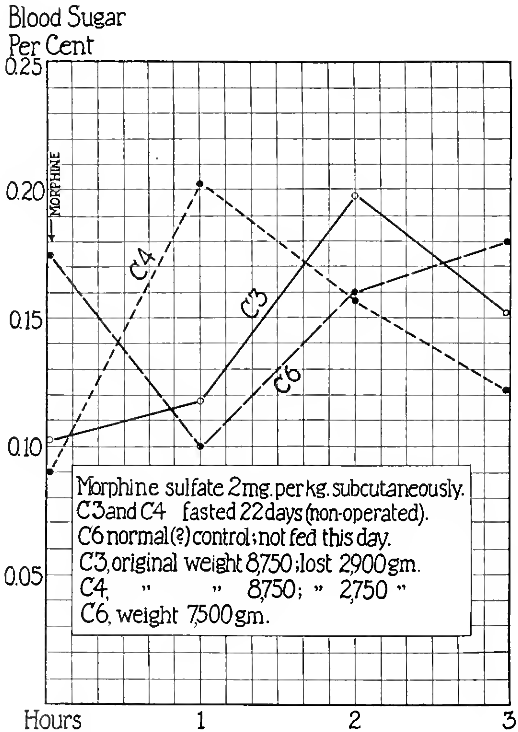
In the fasting animals, Dogs C3 and C4, the morphine produced a considerable rise in the glycemia which was fairly comparable with that observed in the dogs with an experimental pancreatic deficiency. Both dogs, starting at the normal level of 0.090 to 0.104 per cent, showed in 1 to 2 hours after the morphine test, a glycemia of 0.204 and 0.197 per cent, which represent increases of 0.09 to 0.11 per cent. The normal control, Dog C6, showed before the morphine an initially rather high glycemia, 0.175 per cent; 1 hour after the morphine the blood sugar had fallen to 0.10 per cent, but rose again during the next 2 hours to slightly above the original premorphine level. Text-fig. 4 gives these results in graphic form.

The control in this experiment can hardly be considered a normal



TEXT-FIG. 3. Influence of coagulation of the pancreas on morphine glycemia.

animal. The controls in Text-figs. 1 and 2 give a better picture of the response of a normal animal to the subcutaneous injection of morphine.



Text-Fig. 4. Influence of prolonged fasting on morphine glycemia in normal dogs.

In a fifth and final series of experiments we employed dogs in which a pancreatic deficiency had been produced by other means than those used in the previous groups. In Dog BD3, a female weighing 7,750

gm., five-sixths of the pancreas had been resected 3 months before, the residual sixth remaining in connection with the excretory ducts, so that the animal had some pancreatic digestion. 3 days after the operation the blood sugar was 0.277 per cent and the urine showed 0.5 per cent sugar; within a few days, however, the blood sugar fell to a normal level, fluctuating between 0.09 and 0.13 per cent, and the urine was sugar-free. In Dog AK40, a female weighing 11,000 gm., the uncinate process and the tail of the pancreas had been ligated off without resection 2 months before. As there was no reason to expect hyperglycemia or glycosuria in this animal, only one blood sugar examination was made a month after the operation; the result was 0.09 per cent. The urine was not examined. Both dogs, Nos. BD3 and AK40, were in excellent physical condition. The control dogs, Nos. C7 and C8, were apparently normal males weighing respectively 6,250 and 7,000 gm. None of the dogs were fed on the day of the morphine experiment. All the dogs of this group received 1 mg. of morphine sulfate per kilo subcutaneously in the chest.

Text-fig. 5 gives the plotted blood sugar curves of this group. The only animal which shows the characteristically prompt, strong rise in glycemia is the dog with partial pancreatectomy, No. BD3. In this dog the blood sugar rose from 0.125 to 0.213 per cent in 50 minutes, an increase of 0.09 per cent.

In Dog AK40, in which portions of the pancreas had been ligated off without resection, the rise of blood sugar did not exceed 0.03 per cent above the premorphine sample.

The sugar curve of the controls is quite different from that seen in Dog BD3; in Dog C7 the blood sugar rises slowly after 3 hours to 0.05 per cent above the normal level; in C8 the curve exhibits no rise whatever. The latter animal shows an initial fall of blood sugar after the injection of morphine, but in this instance the blood sample had been taken earlier than in the other dogs. A similar initial fall of the blood sugar after morphine may also be seen in Dog C1 of Text-fig. 1 where a blood sample was taken 30 minutes after the morphine dose.

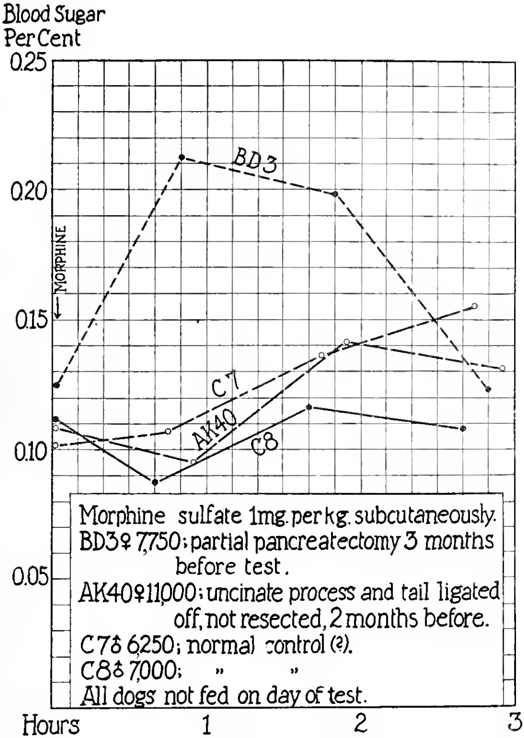
Urine.—The urine of Dogs AK5, AK23, and AK32 (experiments of Text-figs. 1 and 3) was collected for at least 12 hours after the morphine injection. No sugar was found, except in Dog AK5 (Text-fig. 1) where examination revealed

TABLE I.

Blood Sugar Per Cent before and after Morphine.

Time.	Text-fig. 1.				Text-fig. 2.				Text-fig. 3.		Text-fig. 4.			Text-fig. 5.			
	Dog AK5	Dog AK32	Dog C1	Dog C2	Dog AK37	Dog AK5	Dog C3	Dog C4	Dog AK23	Dog C5	Dog C3	Dog C4	Dog C6	Dog AK40	Dog BD3	Dog C7	Dog C8
Before morphine......	0.130	0.192	0.112	0.108	0.109	0.111	0.101	0.090	0.200	0.136	0.104	0.090	0.175	0.109	0.125	0.104	0.114
After ½ hr............	0.206	0.230	0.097	0.111	—	—	—	—	—	—	—	—	—	—	—	—	—
" 1 "	0.246	0.270	0.131	0.133	0.238	0.171	0.128	0.144	0.267	0.137	0.118	0.204	0.100	0.096 (55 min.)	0.213 (50 min.)	0.107 (45 min.)	0.087 (36 min.)
" 2 hrs...........	0.275	0.315	0.128	0.151	0.297	0.209	0.124	0.138	0.306	0.161	0.197	0.157	0.160	0.141	0.197	0.136	0.115
" 3 "	0.240	0.322	0.119	0.127	0.227	0.210	0.106	0.123	0.241	0.155	0.152	0.134	0.181	0.130	0.124	0.157	0.109

0.52 per cent sugar in 160 cc. of urine; there was no albumin. The next 24 hour quantity of urine was sugar-free, although 28 gm. of glucose were fed daily beginning with the day after the morphine test. The urine of the other dogs, except



TEXT-FIG. 5. Influence of partial pancreatectomy on morphine glycemia.

Dog BD3 (Text-fig. 5), was not collected. Dog BD3 showed no sugar in the 18 hour urine collected after the morphine test.

Dosage.—The dose of morphine used in these experiments is in all probability larger than necessary. The glycemia curves of Dog AK5 in Text-fig. 2 and of

Dog BD3 in Text-fig. 5 show that a well marked rise in the blood sugar of a dog with pancreatic deficiency may be obtained with only 1 mg. of morphine per kilo. Possibly a dose of morphine can be found which will increase the blood sugar of prediabetic dogs and have no effect on the blood sugar level of normal animals.

How this hyperglycemia after morphine in dogs with a pancreatic deficiency is produced we shall not discuss here. That morphine in larger doses may cause hyperglycemia in dogs is well known.[3]

General Behavior.—The two groups of dogs of all series exhibited no marked differences in their general response to the morphine injection. All became more or less drowsy; defecation was caused in almost all animals, but retching and vomiting was practically absent in the dogs with pancreatic deficiency and in the fasted controls.

DISCUSSION.

The series of experiments briefly described and figured in the preceding pages show unmistakably that the subcutaneous injection of 1 to 2 mg. of morphine sulfate per kilo of body weight produces a much greater and prompter increase in the blood sugar of dogs with reduced amounts of pancreatic tissue than in normal animals. Text-figs. 1, 2, 3, and 5 and Table I illustrate this well and show the quantitative values obtained in the two classes of dogs.

There is, however, an aspect to this morphine hyperglycemia which may be of practical importance. These dogs with a small or minimal amount of pancreatic tissue may legitimately be considered in a prediabetic stage in the light of much experimental work, especially that of Allen.[4] On this basis, the morphine glycemia test may be of value to the clinician for detecting patients with a weakened carbohydrate metabolism, thus permitting the early institution of an appropriate dietary in order to prevent the potential diabetes from developing into actuality. The test, moreover, is easily carried out, as less than 1 cc. of blood will be necessary if the Epstein method[5] is employed. Only three samples of blood, each 0.2 cc. in amount,

[3] Hirsch, E., and Reinbach, H., *Z. physiol. Chem.*, 1914, xci, 299–301, Experiments VIII, XI, and XIV.

[4] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913.

[5] Epstein, A. A., *J. Am. Med. Assn.*, 1914, lxiii, 1667. A simplification of the procedure is being developed by one of us which will be ready for publication shortly.

would then be necessary, the normal, control sample, and two further samples taken 1 and 2 hours respectively after the morphine injection. The amount of morphine given to the human subject cannot, of course, be calculated kilo for kilo from the doses used for dogs; probably 20 mg. of morphine sulfate ($\frac{1}{3}$ grain) would suffice for an adult.

Whether the morphine test will yield the same result with human beings in the prediabetic stage which we obtained experimentally in dogs, only actual trial can determine. Such a trial, however, we believe warranted by our results and by the simplicity of the procedure. The injection of a moderate dose of morphine is surely not more of a strain to the organism with a possibly defective carbohydrate metabolism than the ingestion of 100 to 200 gm. of glucose; moreover, it will be remembered that morphine has been and is administered to diabetics with apparently beneficial results. Thus, for example, Pavy[6] reported that opium, morphine, and especially codeine reduce the glycosuria in human diabetes. It is therefore unlikely that morphine will work harm in the prediabetic stage of human diabetes.

When to suspect the prediabetic stage in a patient will offer no difficulties to the physician. The combination of racial or family predisposition, neurotic temperament, rheumatoid pains, and periods of muscular weakness point to a possibly defective carbohydrate metabolism, though not associated with a glycosuria. If there is furunculosis, or pruritus, or increased thirst and micturition, or early development of impotence, a prediabetic stage is to be suspected, even though the urine is sugar-free. In such cases the morphine test is worthy of a trial.

Sugar Tolerance.—It should be mentioned that the sugar tolerance of the dogs in which the pancreas had been coagulated by alcohol was surprisingly good. For example, Dog AK5, 90 days after operation excreted only 0.3 gm. of sugar per kilo after being fed 10 gm. per kilo. From the 119th day to the 175th day the same dog was fed daily, except on the day of the morphine test, 4 gm. of sugar per kilo,

[6] Pavy, F. W., *Guy's Hosp. Rep.*, 1870, xv, series 3, 420. It may be noted that as much as $2\frac{3}{4}$ grains (165 mg.) of morphine hydrochloride were administered three times a day to Pavy's Case 3 (p. 430).

in addition to the regular mixed diet, but no sugar appeared in the 24 hour urines.

Another example is furnished by Dog AK32. This dog showed a severe diabetes during the 1st week after the operation: the glycosuria varied between 2.7 to 4.8 per cent and the blood sugar ranged from 0.16 to 0.32 per cent. Within 2 weeks the urine became sugar-free and the glycemia oscillated between 0.09 and 0.17 per cent. A tolerance test on the 21st day after operation (10 gm. of sugar per kilo *per os*) caused no sugar excretion whatever. Thereafter this animal's urine up to the time of death, 118 days after operation, never showed any sugar, beyond an occasional faint trace. It is therefore evident that a strong hyperglycemia after small doses of morphine can even then be obtained when the carbohydrate metabolism is only moderately impaired.

Fasting.—In dogs which had fasted sufficiently long, the subcutaneous injection of 2 mg. of morphine sulfate per kilo sufficed apparently to bring on a definite hyperglycemia. This is shown in Text-fig. 4. In the two animals, Dogs C3 and C4, which had fasted 22 days, the subcutaneous injection of 2 mg. of morphine sulfate per kilo produced in 1 to 2 hours a hyperglycemia of 0.20 per cent, the normal level of the same animals being 0.09 to 0.10 per cent. Since the dogs with deficient pancreas, excepting Dog BD3 (Text-fig. 5), were losing weight constantly in spite of a liberal mixed diet, because they were devoid of pancreatic digestion, it might be objected that the morphine hyperglycemia which we have described merely indicates a fasting state and not a weakness of the carbohydrate metabolism, as we have assumed. This interpretation, however, is probably only partially correct at best and by no means decreases the value of our results. It has been well known since the time of Claude Bernard that fasting or cachectic animals in general may respond with a transitory glycosuria to the ingestion of a full meal of carbohydrates, but it is clear that such an alimentary glycosuria must be due to a temporarily weakened carbohydrate metabolism, for the normal organism would show a sugar-free urine. Therefore the morphine hyperglycemia which we observed during fasting is additional evidence for the correctness of our working hypothesis that morphine

will cause a greater hyperglycemia in an animal with impaired carbohydrate metabolism than in a normal individual.

On the whole, therefore, it may be said that the morphine hyperglycemia during severe fasting is not only no evidence against the correctness of our hypothesis but is, on the contrary, just what that view demands. Furthermore, it must be emphasized that a pancreatic deficiency without obvious fasting, as in Dog BD3 (Text-fig. 5), also causes a well marked hyperglycemia when a small dose of morphine is injected subcutaneously; severe fasting *per se*, therefore, is also not a necessary factor for the appearance of a marked hyperglycemia after morphine in prediabetic dogs.

It should be observed that a moderate degree of fasting is insufficient to bring out the morphine hyperglycemia. Thus a fasting period of 8 days in Dogs C3 and C4 did not cause a marked hyperglycemia after morphine, as the curves of Text-fig. 2 show.

SUMMARY.

The subcutaneous injection of 1 or 2 mg. of morphine sulfate per kilo subcutaneously in dogs with a pancreatic deficiency, whose sugar tolerance is still good, produces a rise in the glycemia about four times greater than the same amount of morphine calls forth in normal dogs.

As dogs with a pancreatic deficiency due to coagulation or partial resection of the gland may legitimately be considered in a prediabetic state, the inference is warranted that the morphine test may be of value in detecting a weakened carbohydrate metabolism in the human subject. The test could easily and without danger be carried out with the micro methods now available for the quantitative determination of blood sugar.

The experimental facts described in this paper give additional corroboration to the view that the response of a normal and of a pathologically altered organism to the same drug in the same dosage may be quantitatively very different.

STUDIES OF LUNG VOLUME. I.

Relation between Thorax Size and Lung Volume in Normal Adults.

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Plates 3 and 4.

(Received for publication, September 15, 1917.)

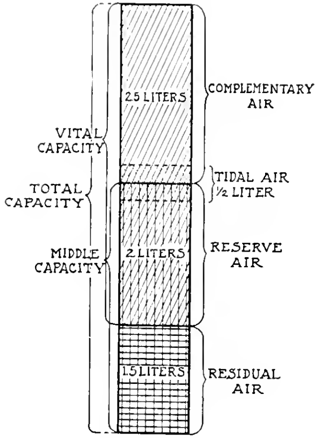
Definition of Terms.

The amount of air a person is able to expire after a maximum inspiration is called "vital capacity" (Hutchinson, 1846). The vital capacity does not, however, indicate all the air within the lungs. A certain quantity remains even after a maximum expiration; we call this "residual air" (Davy, 1800). The sum of the vital capacity and the residual air, *i.e.*, the total volume of air held by the completely filled lungs, is called the "total capacity" or "total lung volume."

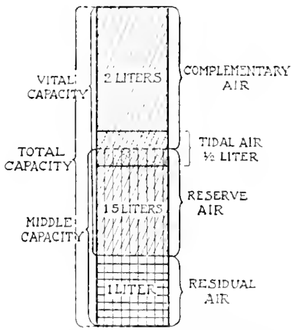
If one stops breathing half-way between a normal inspiration or a normal expiration, there will be in the lungs a certain quantity of air greater than the residual air and less than the total capacity (Text-figs. 1 and 2). We call this amount of air the "middle capacity"[1] (Panum, 1868). The difference between the middle capacity and the total capacity (all that can be breathed in after a half expiration) is called the "reserve air."[2] The difference between middle capacity and residual air (all that can be breathed out after a half expiration) is called the "complementary air."

[1] Siebeck suggested in 1910 defining the middle capacity as the amount of air in the lungs after a full normal expiration, instead of after a half expiration. In this paper the definition of Panum is used.

[2] Hutchinson (1846) created the terms "complementary air" and "reserve air." He used different definitions, however, defining the complementary air as the quantity of air a person can inspire after a normal inspiration, and the reserve air as the amount that can be expired after a normal expiration.



TEXT-FIG. 1. Approximate lung volumes for average normal man.



TEXT-FIG. 2. Approximate lung volumes for average normal woman.

In accordance with the definition now in use, the vital capacity is equal to the sum of the reserve and complementary air. Under normal conditions the difference between the inspiration and expiration (the tidal air) is much less than the vital capacity, and can approximately be estimated at 500 cc. This means that a person only uses 250 cc. of his reserve air and 250 cc. of his complementary air in normal breathing. The rest of the vital capacity is to be considered as a reserve which can be used if necessary under abnormal conditions. There is a striking contrast, however, between the reserve air and the complementary air, the former always being within the chest and the latter always being outside the chest under normal resting conditions (Hutchinson).

Methods for Determining Lung Volumes.

The vital capacity, the tidal air, the reserve air, and the complementary air can be determined by means of a calibrated, easily movable spirometer. In determining the residual air, however, it is necessary to apply a more complicated method. It is usually determined by having the subject expire completely until only the residual air is left in the lungs. He then inspires from a bag or spirometer containing a known amount of nitrogen, oxygen, or hydrogen, which he mixes with the air in his lungs by respiring from five to seven times. Then the mixture is analyzed and the amount of residual air calculated from the degree to which the air in the chest has diluted the gas in the bag or spirometer. The total capacity and the middle capacity can be determined either directly by the bag alone, or indirectly by adding the residual air to the vital capacity and the reserve air respectively, as determining with a spirometer. We have, in our work, determined all the figures by means of the mixing method and later on checked the vital capacity by means of a spirometer.

Our technique has been the following: A 4 liter rubber bag is evacuated and filled with 2 liters of pure oxygen; in determining the residual air we sometimes use 3 liters. The bag is connected to a three-way stop-cock. The subject closes his lips air-tight around the rubber mouthpiece of the stop-cock. The nose is closed by a clamp. For a few respirations the stop-cock is held in such a position as to permit

free passage between the lungs and the outside air. Then the subject brings his lungs to the desired position and retains that position long enough to have the stop-cock turned to connect the rubber bag with his lungs. Four to five fairly deep respirations are sufficient to mix the air in the lungs with the air in the bag (see below). A sample is then drawn out of the bag and analyzed for nitrogen, carbon dioxide and oxygen being absorbed simultaneously by alkaline pyrogallol. The lung volume is calculated in the following way:

$$x \frac{v}{100} = (x + a) \frac{y}{100}$$

$$x = \frac{v - y}{ay}$$

x = the lung volume in liters.

v = the percentage of nitrogen in the lung air before the experiment (usually 79.1 per cent, see page 72).

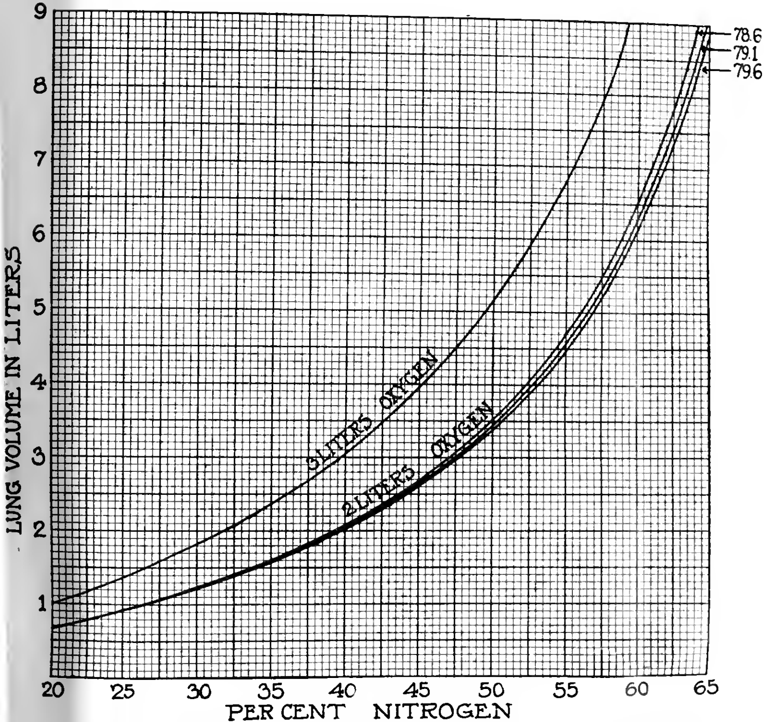
y = the percentage of nitrogen in the sample from the bag at the end of the experiment (or the percentage of nitrogen in the lungs after mixing).

a = the amount of oxygen in the bag in liters at the beginning of the experiment.

It is more convenient to calculate the lung volume by means of the curve in Text-fig. 3.

The curve and formula can only be used if the oxygen in the tank is pure or if a correction is made so that the bag will contain 2 liters of oxygen. In that case the excess nitrogen in the bag, as an impurity in the oxygen, must be subtracted from the calculated lung volume.

Our spirometer is an easily movable Krogh (1912) apparatus. The person is connected with the spirometer by means of a three-way stop-cock of at least 1 sq. cm. bore. In order to determine the vital capacity, the person must fill his lungs and stop breathing for a moment. The stop-cock is then turned and a maximum expiration is made into the spirometer. This is repeated until a constant value is obtained. The determination of the vital capacity can be combined with the determination of the residual air if a three-way stop-cock is so arranged that after expiring into the spirometer the subject is instantly connected with a rubber bag containing 2 or 3 liters of oxygen, as described above. The vital capacity can also be determined by a



TEXT-FIG. 3. Curves for calculation of lung volume (air content) as determined by the dilution method. The upper curve is for use when 3 liters of pure oxygen are mixed with the lung air, the lower when 2 liters of oxygen are used. The lower curve is given in three forms to indicate the range of error which may be caused by maximum variations in the nitrogen content of the alveolar air from the usual value of 79.1 per cent.

maximum inspiration from the spirometer, but the results obtained are somewhat smaller than those obtained by expiration (Table III).

In order to determine the reserve air and the complementary air, the person must breathe normally for some time into the spirometer, which contains 3 or 4 liters of 50 per cent oxygen to prevent dyspnea. When the breathing is regular, a maximum respiration is made. This respiration must equal the previously found vital capacity.

The figures can be given directly or in values corrected for temperature, pressure, and moisture. We have not corrected them. Our figures refer to the gas volumes measured at 21° ± 3°C.

Accuracy of Methods.

Spirometry.—The spirometer method will always give the true vital capacity at that particular moment. The maximum reading error is 50 cc. Measuring the vital capacity of the same person several times, one finds that the results obtained differ by amounts usually less than 200 cc., in most cases from 3 to 6 per cent of the vital capacity. These differences are not due to the method, but to the inability of the subject to reach the same point in inspiration or expiration, or both, every time. Bohr recognized the fact that the vital capacity is not constant. He considered the maximum inspiration as a fixed point, and that the discrepancies in the determinations of the vital capacity were due to the expiration, the last part of which is done by the diaphragm. Hasselbalch showed later that it is possible to train a person to increase his total lung volume. He found, furthermore, that the total capacity and vital capacity in three normal persons decreased when they changed from the standing to the lying position. This does not exclude the fact that the maximum inspiration is a fixed point and the maximum expiration a variable point when the determinations are made within a short time and with the subject in the same position.

Dilution Method.—In the rubber bag method, or, as it may more accurately be called, the dilution method,[3] there are possibilities for several errors. The analytical error is very small because a large

[3] The volume of air in the lungs is determined in a way analogous to the determinations of the residue in the stomach in Ewald's test meal.

amount of air may be taken for analysis. In a determination where 30 cc. are taken for analysis, the error falls below 0.2 per cent, even when no special precautions are taken, such as the use of a thermobarometer. The main source of error is the difficulty in obtaining a homogeneous mixture of air in the lungs and in the bag. It is generally supposed that five to seven fairly deep respirations are sufficient to mix even pure hydrogen with the air in the lungs. A recent study by Sonne has shown that it is essential to pay more attention to that problem than was formerly considered necessary. Sonne found that it was very difficult, and in some instances almost impossible, to get a homogeneous mixture in the lungs by inhaling foreign air. He found it extremely difficult to get a proper mixture by mixing the lung air with nitrous oxide as is done in Krogh and Lindhard's (1912) method for determining the blood flow. Krogh and Lindhard themselves have later (1917) admitted this difficulty. We have, therefore, been very careful in controlling our results. We have done this in three different ways:

(1) By performing our experiments on the same person with different numbers of respirations (Table I). As seen in the table, increasing the number of respirations beyond four of at least 2 liter excursions does not change the results. The slight differences obtained

TABLE I.

Effect of Variations in the Number of Respirations on Results by the Dilution Method.

Name.	Position of chest.	Excursion of respiration.	Lung volume calculated from analyses of mixed gases after varying number of respirations.							
			1	2	3	4	5	6	7	8
		liters	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*
Dr. F.	Maximum expiration.	2.0	1.46	1.70	2.02	1.98 1.84				
" A.	" inspiration.	3.5				5.75				5.58
" A.	Normal "	3.0					3.85	3.96		3.90 4.00 3.92
" S.	Maximum "	3.0					6.27			6.20
" P.	" expiration.	2.0	2.16 2.20	2.45	2.49	2.50 2.74	2.50			

are not due to an incomplete mixture, but to the previously mentioned impossibility of starting the respiration from the same point in different experiments. We have tried to overcome this difficulty in (2).

(2) By taking samples from the rubber bag after a different number of respirations in the same experiment (Table II). These experiments show that we obtained almost constant values of nitrogen in the rubber bag after four or five respirations. In all the experiments in this table the subject has started from a maximum inspiration, which probably is a more unfavorable condition for mixing than starting from a maximum expiration, because one is unable to empty

TABLE II.

Analyses of Mixed Gases in the Bag after Varying Numbers of Successive Respirations.

Name.	Position of chest.	Amount of pure oxygen in bag.	Excursion of respirations.	Sample 1.		Sample 2.	
				No. of respirations.	Nitrogen.	No. of respiration.	Nitrogen.
		liters	*liters*		*per cent*		*per cent*
Dr. V.	Maximum inspiration.	2 (approximately)	3½	4–6	60.5	7	60.0 60.1
" V.	" "	2	3½	4–6	59.1	8	59.4
" L.	" "	2 (approximately)	3	4–5	58.2	7	58.5
" S.	" "	2	3½	4–6	57.4	8	57.5
" S.	" "	2 (approximately)	3½	4–6	58.3	7	58.5

the rubber bag each time. The respiratory excursions in these experiments have been from 3 to 3½ liters.

(3) By determining the vital capacity as the difference between the total lung volume and the residual air, determined by the dilution method, and checking this by determining the vital capacity with the spirometer. We have done this in all but two of our subjects. The results are shown in Table III.

The values for the vital capacities determined by expiration into the spirometer are, with few exceptions, from 1 to 5 per cent greater than the values obtained by the dilution method. The reason for this is undoubtedly that we have given as our spirometer values the highest figures obtainable with the spirometer, whereas the dilution method figures are the average of all determinations. The values

for the vital capacities determined by inspiration from the spirometer are always slightly smaller than the values obtained by expiration. This is probably accounted for by the greater power of the expiratory muscles and by the resistance of the spirometer. Another reason may be the difference in temperature and moisture content of the expiratory air.

Inconstancy of the Nitrogen Percentage in the Lung Air.—Another possible error is due to the inconstancy of the nitrogen in the lung air and the impossibility of determining it in relation to the determination of the lung volume. We determined the nitrogen percentage in a sample from the total amount of expired air in six normal people. Six determinations were done on each person, three on the expired air after an ordinary expiration and three after a maximum inspiration. The values fell between 78.7 and 79.5 per cent in 27 cases; in 9 cases the values fell outside these limits but within 78.4 and 79.6 per cent. The variations in the same person are usually as great as from person to person. We have used 79.1 per cent in all our calculations. The curves in Text-fig. 3 show the limits of the possible errors. The constancy of the figures in Tables I and II shows that the actual errors due to increased absorption of oxygen in blood plasma and tissues from the oxygen-rich mixture breathed during the 10 to 15 seconds of the experiment, to the excretion of nitrogen from blood, and to the deviation of the respiratory quotient from 1.0 are negligible.

Standard Procedure for the Determination of Lung Volumes.

The determinations of the lung volume in Subject 2 (Table III) were done in a way which we considered the best and most reliable. It is given in detail as follows:

(1) *Determination of Vital Capacity by Means of Expiration in the Spirometer.*—

Expiration.	Volume. *liters*	Temperature. *°C.*	Pressure. *mm.*
First.............	5.75	24	762
Second...........	5.80		
Third............	5.95		
Fourth...........	5.95		

Vital capacity = 5.95 uncorrected.

(2) *Determination of Residual Air by the Dilution Method.*—The subject breathed through a three-way stop-cock by means of which he could be connected with either the spirometer or a rubber bag containing 3 liters of oxygen. He filled his lungs with room air and breathed repeatedly into and out of the spirometer. When the volume of expiration equalled the previously determined maximum vital capacity (5.95 liters), the stop-cock was turned in such a manner that the next inspiration was made from the bag. The oxygen drawn in from the bag was rebreathed seven times in 15 seconds. The nitrogen content of the mixed gases was 32.6 per cent, indicating a residual air volume of 2.1 liters.

(3) *Total Capacity.*—The total capacity was then determined with the bag, which contained 2 liters of oxygen. The subject respired eight times. The nitrogen content of the mixture was 63.4 per cent, indicating a total capacity of 8.05 liters.

Vital capacity determined by spirometer = 5.95 liters.
Vital capacity determined by bag = 8.05 − 2.1 = 5.95 liters.

(4) *Middle Capacity.*—While breathing quietly the subject was connected with a spirometer containing about 4 liters of air with 50 per cent oxygen. He continued regular breathing from the spirometer, which registered as follows:

Readings of the Spirometer Dial.

	After inspiration. *liters*	After expiration. *liters*	Mean. *liters*	Vital capacity. *liters*
Normal respiration.....	3.2	3.9	3.55	
	3.1	3.9	3.50	
	3.15	3.85	3.50	
Maximum "	0.4	6.1	—	5.7

Reserve air = 6.1 − 3.5 = 2 6 liters.
Complementary air = 3.5 − 0.4 = 3.1 liters.

The value for the vital capacity obtained in this experiment is 6.1 − 0.4 = 5.7, instead of 5.95. The reason for this is that the maximum inspiration was made from the spirometer, a condition which, as mentioned before, regularly gives a smaller vital capacity than that registered when the lungs are filled from the free air (Table III). For this reason we increase the value of the complementary part of the total air by 0.25 to 3.35 liters.

Immediately afterwards the chest measures of the subject were taken.

Lung Volumes in Eighteen Normal Individuals.

The results of our experiments on eighteen normal persons between 20 and 38 years of age are tabulated in Table III, and diagrams of the

TABLE III.

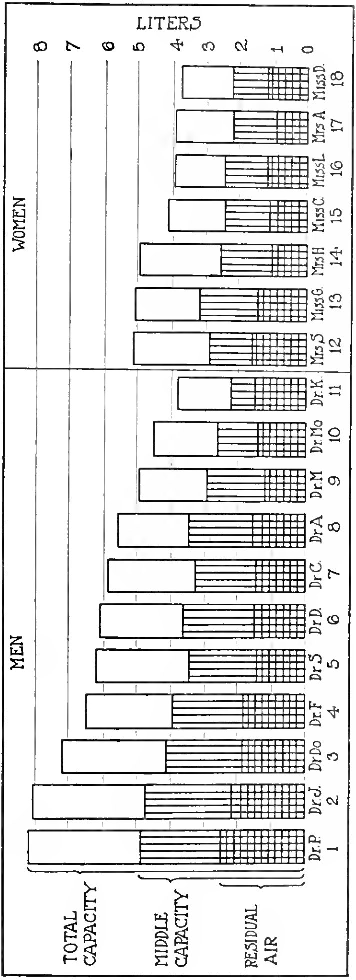
Summary of Lung Volume Determinations on Normal Individuals.

No. of individual.	Name.	Age.	Height.	Weight.	Bag.						Spirometer.	
					Residual air.	Middle capacity.	Total capacity.	Vital capacity.	Reserve air.	Complementary air.	Vital capacity. Expiration.	Vital capacity. Inspiration.
		yrs.	*cm.*	*kg.*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*	*liters*
1	Dr. P.	34	185	90	2.48	4.80	8.22	5.74	2.32	3.42	5.90	5.70
2	" J.	31	179	76	2.10	4.70	8.05	5.95	2.60	3.35	5.95	5.70
3	" Do.	29	186	86	1.86	—	7.20	5.34	—	—	—	—
4	" F.	32	178	68	1.87	3.89	6.51	4.64	2.02	2.62	4.90	4.85
5	" S.	32	178	91	1.47	3.45	6.24	4.77	1.98	2.79	4.87	4.80
6	" D.	34	178	69.5	1.52	3.66	6.13	4.61	2.14	2.47	4.65	4.60
7	" C.	29	172.5	65	1.46	3.31	5.88	4.42	1.85	2.57	4.63	4.55
8	" A.	38	165	67	1.61	3.50	5.58	3.97	1.89	2.08	3.87	3.77
9	" M.	32	167.5	63	1.25	2.99	4.95	3.70	1.74	1.96	3.97	3.93
10	" Mo.	29	162.5	51	1.41	2.60	4.56	3.15	1.19	1.94	3.40	3.35
11	" K.	28	152	52	1.53	2.23	3.84	2.31	0.70	1.61	2.40	2.35
12	Mrs. S.	34	175	88	1.64	2.88	5.10	3.46	1.24	2.22	—	—
13	Miss G.	26	173	63	1.42	3.17	5.05	3.63	1.75	1.88	3.75	3.70
14	Mrs. H.	24	162	59	1.07	2.69	4.91	3.84	1.62	2.22	3.90	3.85
15	Miss C.	23	169	65	1.10	2.40	4.12	3.02	1.30	1.85	3.15	3.10
16	" L.	29	156	43	1.15	2.45	3.93	2.78	1.30	1.48	2.75	2.70
17	Mrs. A.	28	158	52	0.97	2.28	3.93	2.96	1.31	1.65	3.10	2.95
18	Miss D.	21	160	53	1.15	2.35	3.72	2.57	1.20	1.40	2.65	2.60

same determinations are shown in Text-fig. 4. The values for the different lung volumes in these determinations agree with what other investigators have found.

From his determinations on eight normal men and four normal women, Bohr derived a standard for the different lung volumes which is usually accepted.

We have divided our results into two groups according to the



TEXT-FIG. 4. Chart showing the lung volumes in eleven normal men and seven normal women.

TABLE IV.

Average Lung Volumes for Normal Men and Women in Standing Position.

Sex.	Residual air.	Reserve air.	Mean.	Complementary air.	Total capacity.
	liters	*liters*	*liters*	*liters*	*liters*
Men	1.5	2.0	3.5	2.5	6.0
Women.	1.0	1.5	2.5	2.0	4.5

sexes and believe that the figures given in Table IV and Text-figs. 1 and 2 represent the approximate average somewhat more closely than the approximations used by Bohr; namely, 1 liter of residual, 2 liters of reserve, and 2 liters of complementary air.

Previous Investigations to Find a Relationship between the Vital Capacity and Other Body Figures.

Pulmometry has never played an important part in clinical medicine, chiefly on account of the great variations in the lung volumes of different persons. For that reason we have been unable to tell whether the lung volume in a pathologic case is normal or not for the individual examined unless the deviation from the usual values is great. The variations in the values of the lung volume[4] in different individuals have been recognized by even the earliest investigators.

Borelli (1679) was the first who tried to determine the air in the lungs. He found that from 300 to 600 cc. are taken in by a single inspiration. Jurin (1718) says about Borelli's figures: "But this quantity is different not only in different persons, but even at different times in one and the same person." Hales (1728) determined the air in the lungs to be 4 liters. Goodwyn (1788) says after reporting his own experiments: "These experiments are sufficient to show that the lungs contain a considerable quantity of air, even after complete expiration, but this quantity must vary in different subjects in proportion to the capacity of the thorax. It is, therefore, extremely difficult to establish a medium. However, we shall for the present adopt the medium quantity of these latter experiments and say that the lungs of the human subject contain 109 cubic inches (1,800 cc.)[5] of air after complete expiration."

[4] It took a considerable time before the differences between vital capacity, residual air, and so forth were recognized.

[5] Determined post mortem by filling the pleural cavities with water, in this way compressing the lungs (the diaphragm was fixed).

Davy (1800), who invented the dilution principle in determination of the residual air, gives the figures for his own lungs: "So that making the corrections for temperature, it would appear, that my lungs in a state of voluntary inspiration, contained about 254 cubic inches (4,160 cc.); in a state of natural inspiration, about 135 (2,210 cc.); in a state of natural expiration, about 118 (1,190 cc.); and in a state of forced expiration 41 (670 cc.)." He also remarks: "This capacity is most probably below medium; my chest is narrow, measuring in circumference but 29 inches." Hutchinson, by the invention of the spirometer, made the easily determinable vital capacity the central point in the pulmometry until the time of Bohr, half a century later. Hutchinson realized that, should the vital capacity be of any importance in clinical medicine, it was necessary to find some relationship to other body figures. For that purpose he examined 1,012 normal men and women and found that there was a certain relationship between the height and the vital capacity. He worked out the figures by means of which it should be possible to calculate the normal vital capacity from the height of the person. He showed that the weight, age, and sex might modify these figures to a slight extent. Between the vital capacity and the circumference of the chest he could not find any proportion at all. Simon (1848) confirmed Hutchinson's results as far as height was concerned, but he found that if only lean persons were used, there was some relation between the vital capacity and the circumference of the chest. Fabius (1853) found a rather close relation between the vital capacity and the volume of the trunk, which he calculated from the circumference of the chest and the distance from the neck (eminentia occipitalis) to the tip of the os coccygis. His first idea was to compare the vital capacity with the "chest volume"; he gave that up because he was unable to obtain any figures for the chest volume. Apparently without knowing Fabius' publication, Müller (1868) and Schönfeld (1882) found the same thing. The idea of Fabius (Müller and Schönfeld) did not attract much attention and was never used by others in clinical medicine. Hutchinson's old idea of calculating the vital capacity from the height prevailed and was generally used, sometimes with a slight modification of the constant and sometimes in combination with a correction for weight or age (Schneevoogt, 1854).

Wintrich (1854), Arnold (1855), von Ziemssen (1888), Cornet (1907), and Peabody (1917) have all adopted the principle laid down by Hutchinson, even if they have used it in a somewhat different way. Von Ziemssen, for instance, used a quotient (1:20 in men and 1:17 in women) to express the ratio between vital capacity and height. This quotient (Ziemssen's quotient) has been used in several papers, particularly in papers dealing with the vital capacity in people suffering from tuberculosis. Peabody divides his patients (heart patients) into four groups, according to the height.

A relationship between the residual air and other body factors has not been worked out. Only some rather rough estimates have been adopted (Schenck, 1894).

Bohr was the creator of a new era in pulmometry. He objected to the use of Hutchinson's figures for estimating the normal vital capacity from the height

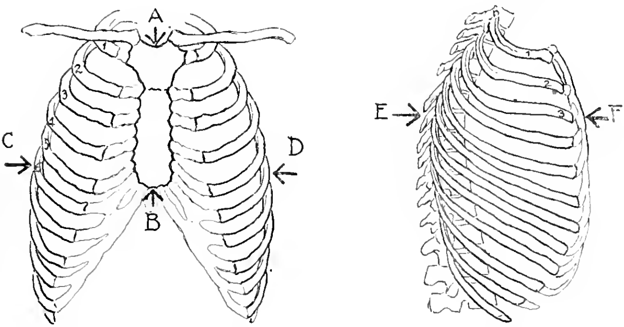
because the individual variations are too great. He furthermore objected to exclusive use of the vital capacity, because it does not take the total or residual air into consideration. He and his pupils (Hasselbalch, Rubow, Siebeck, and Bie and Maar) in several publications investigated the relation between the total lung volume, the middle capacity, and the residual air under normal and pathologic conditions, and put less stress on the absolute figures than on their relationships. The problems raised by these investigators have attracted attention for a good many years.

Determination of the "Chest Volume" and Calculations of a Ratio between Chest Volume and Lung Volume in Different Positions of the Chest (Full Inspiration, Rest, and Full Expiration).

The problem of finding an accurate relationship between the lung volume and chest or body size remained unsolved. It seemed to us, however, that it might be possible to approach the solution by using the chest volume as the constant and the lung volume as the variable. The reason for this seems obvious when we consider the chest wall as a sort of spirometer. When a person is respiring from a spirometer, it is a natural thing to look upon the chest wall as another spirometer connected with the first one and moving inversely to the latter. And if we take different individuals it is natural to regard their chests as spirometers containing different amounts of air.

The x-ray pictures (Figs. 1 and 2) illustrate fairly well what we mean. Fig. 1 (No. 7, Table III) is from the same person with the chest in full inspiration and expiration. Fig. 2 is from two different persons (Nos. 1 and 11) and shows the possibilities of individual variation. Our problem was to find measurements which could be used in calculating the chest volume, which alone seemed a logical basis for calculation of lung capacity. The old idea of using the chest circumference must be given up because the muscles, fat, and breasts give room for a considerable error. It seems obvious that the best way is to consider the chest as a geometrical figure and take three dimensions, the product of which will represent a volume proportional to the chest volume. We have then to measure the height, depth, and breadth of the chest and to do it in such a way that (1) the fat and muscles play as small a part as possible, (2) the different diameters represent parts of the chest wall which move in fair accordance to the respiration.

After some consideration and experiments we came to the following procedure which has been used in all our cases. The height of the chest is taken as the length of the sternum from incisio intraclavicularis to a point just below articulatio sterno-xiphoidea. The depth is then taken as the horizontal distance from the middle of the sternum at the insertion of the third rib to the spinal column, and the breadth is the distance across the sixth ribs in the midaxillary line. The points between which the measures are taken are almost without any muscular covering. The transverse points are in the axilla between



TEXT-FIG. 5. Points on the thorax where the chest measurements are taken. *AB* is the height of the chest, *CD* the breadth, and *EF* the depth.

musculus pectoralis major and musculus latissimus dorsi. The distances representing the depth and breadth vary with the phases of inspiration; the height is constant (Text-fig. 5).

The measuring requires some practice and a good deal of care. We do it in the following way: The person stands in a natural position. The points are found and marked. The point on the upper part of the sternum is rather easy to find; it is just above the edge of the bone. The lower point is sometimes very difficult to locate. We do it as follows: The curvature is found and lines are drawn to indicate it. Then we try to find the joint between the sternum and processus xiphoideus. It is usually slightly prominent. The point from which we measure is just below that prominent ridge. Locating the tip

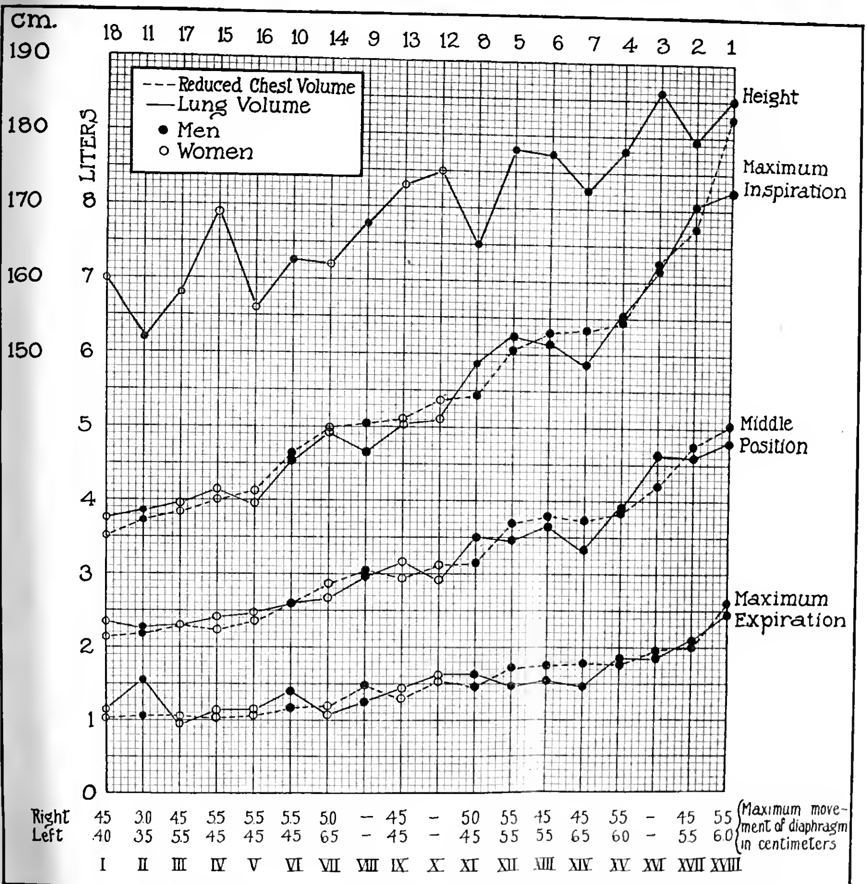
of the processus xiphoideus sometimes helps, but it cannot always be done. The lateral points are easy to find by counting the ribs. In taking the measure it is necessary to put the nodes of the pelvimeter tight to the chest wall in order to get as close to the bone as possible. It is particularly necessary to take care that the ends of the pelvimeter do not slip and go into the intercostal spaces. The first measures are taken in rest (half-way between normal expiration and inspiration, position of middle capacity). The pelvimeter is kept on and the person is requested to take a maximum inspiration and stop a second while the measures are taken. Then he is asked to expire to the residual air and stop for another measurement. The product of the figures obtained does not, of course, represent the real chest volume, but a volume approximately proportional to it.

In Table V are given the data on different normal individuals. The product of the measurements in the three dimensions is given as the chest volume and the ratio between the chest volume and lung volume calculated as

$$\frac{100 \times \text{lung volume}}{\text{chest volume}}$$

The average ratio for the total lung capacity is 55, for the middle capacity 37, and for the residual air 19.

The curves of Text-fig. 6 show the different chest volumes multiplied by these factors (reduced chest volume). It will be seen that there is a close agreement between the reduced chest volumes and the actual lung capacities. We have plotted the height on the upper part of the chart to show that the relations between the different lung volumes and the height are much more variable than the relations between the lung and chest volumes. The relationship between height and vital capacity (Hutchinson) is equally variable. The vital capacity, on the other hand, bears nearly as constant a ratio to the reduced chest volume as do the total or middle capacities. The average ratio between the vital capacity and the middle chest volume is 45 (Table VI).



TEXT-FIG. 6. Chart showing air contents of lungs (lung volumes) in normal subjects as determined in the three respiratory positions by the dilution method (solid lines) and as calculated from the thoracic measurements (broken lines). The subjects are arranged in order according to chest volumes measured at maximum inspiration. The numbers above the chart are those by which the same subjects are designated in Text-fig. 4. The numbers below indicate the maximum excursion of the right and left diaphragm in centimeters, as calculated from fluoroscopic tracings.

TABLE V.

Summary of Thorax Measurements in the Three Positions of Respiration, Namely, Expiration, Rest, and Inspiration, and Ratios of Chest Volumes Calculated from These Measurements to Lung Volumes.

No. of individual.	Name.	Position of chest.	Sternum.	Diameter.		Chest volume.	Lung volume.	Ratio $\frac{100 \times \text{lung volume}}{\text{chest volume}}$
				Ant.-Post.	Transverse.			
			cm.	*cm.*	*cm.*	*liters*	*liters*	
1	Dr. P.	Expiration.	22.0	21.0	29.5	13.63	2.48	18.2
		Rest.	22.0	22.0	31.0	15.00	4.88	32.0
		Inspiration.	22.0	24.0	32.5	17.15	8.22	48.0
2	Dr. J.	Expiration.	21.0	17.5	29.0	10.65	2.10	19.7
		Rest.	21.0	19.5	30.5	12.49	4.70	37.6
		Inspiration.	21.0	21.0	32.0	14.08	8.05	57.2
3	Dr. Do.	Expiration.	20.5	17.0	29.5	10.28	1.86	18.1
		Rest.	20.5	18.5	30.0	11.36	4.63	—
		Inspiration.	20.5	20.0	32.0	13.12	7.20	54.8
4	Dr. F.	Expiration.	19.5	18.5	26.0	9.38	1.87	19.9
		Rest.	19.5	19.0	28.0	10.38	3.89	37.5
		Inspiration.	19.5	20.0	30.0	11.70	6.51	55.6
5	Dr. S.	Expiration.	18.0	18.5	27.5	9.15	1.47	16.1
		Rest.	18.0	19.5	28.5	10.00	3.45	34.5
		Inspiration.	18.0	20.0	30.5	11.00	6.24	56.7
6	Dr. D.	Expiration.	20.5	18.5	24.5	9.30	1.52	16.4
		Rest.	20.5	19.5	25.5	10.20	3.56	35.9
		Inspiration.	20.5	21.5	26.5	11.45	6.13	53.6
7	Dr. C.	Expiration.	19.5	17.5	27.5	9.39	1.46	15.6
		Rest.	19.5	18.5	28.0	10.10	3.31	32.6
		Inspiration.	19.5	20.0	29.5	11.50	5.88	51.1
8	Dr. A.	Expiration.	20.0	16.0	24.5	7.83	1.61	20.6
		Rest.	20.0	17.0	25.0	8.50	3.50	41.2
		Inspiration.	20.0	18.0	27.5	9.90	5.88	56.4
9	Dr. M.	Expiration.	19.5	15.5	26.0	7.86	1.25	16.4
		Rest.	19.5	16.0	26.5	8.26	2.99	36.4
		Inspiration.	19.5	17.0	27.5	9.11	4.64	51.0
10	Dr. Mo.	Expiration.	19.0	15.0	22.0	6.27	1.41	22.5
		Rest.	19.0	16.0	23.0	6.99	2.60	37.4
		Inspiration.	19.0	18.0	24.5	8.38	4.56	54.6

TABLE V—*Concluded.*

No. of individual.	Name.	Position of chest.	Sternum.	Diameter. Ant.-Post.	Diameter. Transverse.	Chest volume.	Lung volume.	Ratio $\frac{100 \times \text{lung volume}}{\text{chest volume}}$
			cm.	*cm.*	*cm.*	*liters*	*liters*	
11	Dr. K.	Expiration.	16 0	14 5	24 0	5.57	1.53	27.5
		Rest.	16 0	15 0	24.5	5.88	2.23	37.9
		Inspiration.	16 0	17 0	25.0	6.80	3.84	56.4
12	Mrs. S.	Expiration.	17.5	18.5	24.5	7.93	1.64	20.7
		Rest.	17.5	19.0	25.2	8.38	2.88	34.3
		Inspiration.	17.5	20 2	27.5	9.72	5.10	52.4
13	Miss G.	Expiration.	18.3	14.8	25.3	6.85	1.42	20.7
		Rest.	18.3	16.5	26.1	7.88	3.17	40.3
		Inspiration.	18.3	18 5	27.3	9.29	5.05	54.2
14	Mrs. H.	Expiration.	17.2	16.0	24.8	6.83	1 07	15.7
		Rest.	17.2	17.1	25.8	7.77	2.69	34.6
		Inspiration.	17.2	18.3	28.3	8.91	4.91	55.1
15	Miss C.	Expiration.	17.9	13 9	22.2	5.52	1.10	19.9
		Rest.	17.9	14 9	22.6	6 03	2.40	39.7
		Inspiration.	17.9	15.7	25.8	7 25	4.12	55.8
16	Miss L.	Expiration.	17.1	14 9	22 5	5.73	1 15	20.0
		Rest.	17 1	16 0	23.4	6.40	2.45	38.3
		Inspiration.	17 1	17.3	25.3	7 49	3 93	52.4
17	Mrs. A.	Expiration.	16 2	14.0	23.5	5.32	0.97	18.2
		Rest.	16.2	15.5	24.5	6.15	2.28	37.1
		Inspiration.	16 2	17.1	25.4	7 04	3 93	55.4
18	Miss D.	Expiration.	17.1	14 0	22 1	5.30	1.15	21.7
		Rest.	17 1	15 0	22.2	5.79	2.35	40.3
		Inspiration.	17 1	16.5	23 2	6.55	3.72	57.8

Excursions of the Diaphragm.

Realizing that the measurement of the chest wall does not give us the variations in the height of the thoracic cavity, we have made a particular study of the movement of the diaphragm by means of x-rays.[6] The figures are given in Table VI. It will be seen that there is one man

TABLE VI.

Maximum Excursions of the Right and Left Diaphragm.

No. of individual.	Name.	Greatest possible movement of diaphragm. Right.	Left.	Right + left.	$\frac{100 \times \text{residual air}}{\text{chest volume at expiration}}$	$\frac{100 \times \text{vital capacity}}{\text{chest volume at rest}}$
				Men.		
		cm.	*cm.*	*cm.*		
1	Dr. P.	5.5	6 0	11.5	18.2	38.3
2	" J.	4.5	5.5	10 0	19.7	47.7
3	" Do.	—	—	—	18.1	47 0
4	" F.	5.5	6.0	11.5	19.9	43.0
5	" S.	5.5	5.5	11 0	16 1	47.7
6	" D.	4 5	5.5	10.0	16 4	45.2
7	" C.	4.5	6.5	11.0	15.6	43.7
8	" A.	5.0	4.5	9.5	20 6	46.7
9	" M.	—	—	—	16.4	44 8
10	" Mo.	5.5	4.5	10 0	22.5	45.1
11	" K.	3.0	3.5	6.5	27.5	39.2
				Women.		
12	Mrs. S.	—	—	—	20.7	41.3
13	Miss G.	4.5	4.5	9.0	20 7	46.1
14	Mrs. H.	5.0	6.5	11.5	15.7	49.4
15	Miss C.	5 5	4.5	10 0	19 9	50 1
16	" L.	5.5	4.5	10 0	20 0	43.5
17	Mrs. A.	4.5	5.5	10 0	18.2	48.1
18	Miss D.	4 5	4 0	8 5	21.7	44.4

[6] We used fluoroscopy. Being unable to use parallel light we worked out a correction for the parallax by measuring the distance from the light to the screen (50 cm.) and the distance from the light to the middle part of the diaphragm (35 cm.). The correction is very close to 0.7 in all instances; for that reason all our directly found values have been multiplied by that factor.

with a very small movement of his diaphragm. His residual air is unusually great (Text-figs. 4 and 6). He apparently expired naturally as far as the thoracic movement was concerned, but was unable to press his diaphragm up at the end of the expiration. He was a physically untrained man, with rather undeveloped abdominal musculature. A too small movement of the diaphragm might, of course, indicate that he was unable to lower it during inspiration. The normal figures for his total capacity and the abnormally high figure for his residual air prove that this was not the case. One of the women (No. 14, Table V and Text-fig. 4) had a particularly small residual ratio, 15.7. It will be seen that the movement of her diaphragm is very extensive. She had been trained in college to breathe very deeply and had powerful abdominal muscles. She wore, like all the other women, a rather loose corset during the determination. The importance of the movements of the diaphragm will be discussed more in a later paper.

SUMMARY.

1. The total capacity, middle capacity, and residual air have been determined on 11 normal men and 7 normal women. All the determinations have been done on subjects in standing position and at least 2 hours after a meal.

2. The figures for the total and middle capacities agree with those of previous investigators, particularly with Bohr's. The values for the residual air seem to be a little higher than those previously published.

3. A procedure has been devised by means of which it has been possible to find a numerical relationship between external chest measurements and lung capacity.

4. With the aid of the relationship thus ascertained the lung capacity normal for a chest of given measurements can be estimated.

5. The excursions of the diaphragm have been studied.

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EXPLANATION OF PLATES.

PLATE 3.

FIG. 1. Two x-ray pictures from a normal man, No. 7, taken after maximum expiration (*a*) and maximum inspiration (*b*). The outline of *a* is superimposed on *b*.

PLATE 4.

FIG. 2. Two x-ray pictures from No. 11 (*a*) and No. 1 (*b*). They are both taken in maximum inspiratory position. *a* is superimposed on *b*.

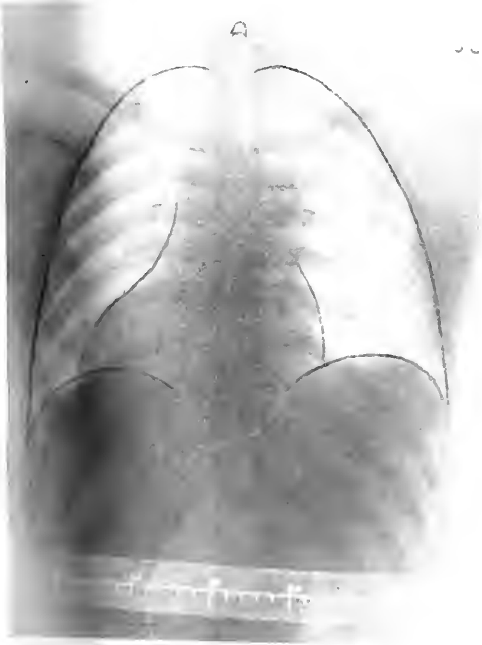

FIG. 1a.

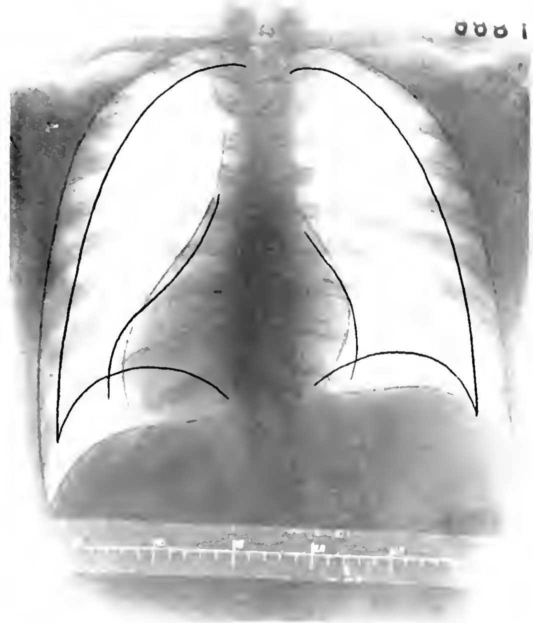

FIG. 1b.

(Lundsgaard and Van Slyke: Studies of lung volume. I.)

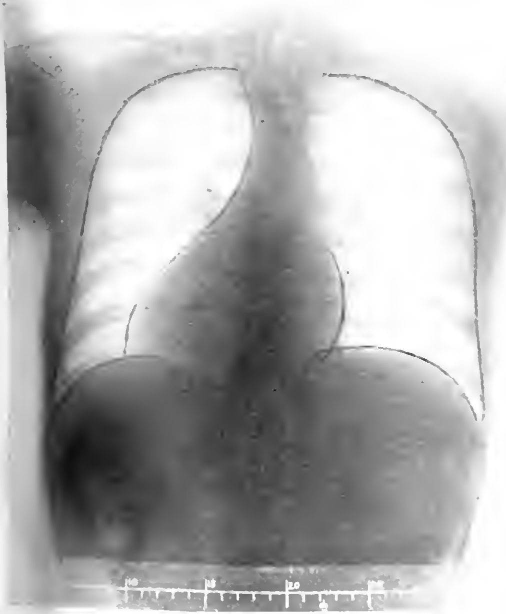

FIG. 2a.

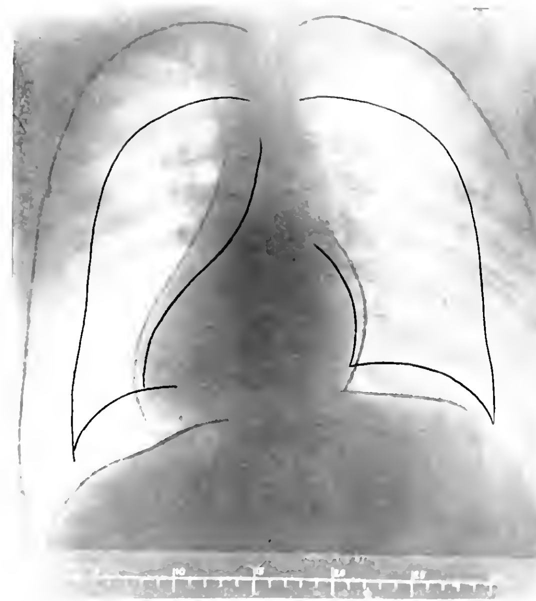

FIG. 2b.

STUDIES OF LUNG VOLUME.

II. Tuberculous Men.

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Hutchinson,[1] who invented the spirometer (1846), determined the vital capacity in twenty-two cases of early and nine cases of advanced pulmonary tuberculosis, and found that the vital capacity was subnormal in all. In the former group he found a decrease of from 10 to 50 per cent, in the latter of from 40 to 80 per cent. He calculated the normal figures from the height of the patient.[2] Since then numerous investigators, for example, Simon (1848), Wintrich (1854), Schneevoogt (1854),[3] Arnold (1855), Faivre (1864), Schönfeld (1882), and Hecht (1885), have confirmed this observation, and since von Ziemssen (1888) introduced his quotient[4] for the relation between height and vital capacity, spirometry has become practically a matter of routine in many clinics.

Despite the mass of data gathered, few attempts have been made to show which of the factors affecting the vital capacity are responsible for its decrease in tuberculosis.

Charlier studied the residual air in a group of patients with pulmonary tuberculosis and found it decreased. Siebeck examined the different lung volumes (total capacity, middle capacity, and residual air) in five patients and found the total capacity diminished, the middle capacity about normal, the residual air increased, and the vital capacity considerably diminished. However, the difficulty in establishing normal figures for a given pathologic case[5] made the de-

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[1] The mere title of Hutchinson's article shows how important he considered the determination of the vital capacity in patients. The title is, "On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer."

[2] For a detailed discussion see Lundsgaard and Van Slyke.

[3] Schneevoogt says: Tuberculosis of the lungs particularly will become apparent in this way before it can be diagnosed by any other means.

[4] Ziemssen's quotient is 1:20 in men and 1:17 in women. That means that 1 cm. of height corresponds to a vital capacity of 20 (17) cc.

[5] Siebeck says (p. 208): Not knowing the normal total capacity for a patient it is difficult to state anything about a deviation from the normal.

termination of absolute figures rather problematic. In other words, we do not know whether the decrease in the easily determinable vital capacity is caused by incomplete expiration (increased residual air) or by lessened inspiration (decreased total capacity). It is the purpose of the present paper partially to fill this lack in our present knowledge.

In a previous paper two of us (Lundsgaard and Van Slyke) have established evidence of a close relation between the dimensions of the chest and the capacity of the lungs in the three main positions,—maximum inspiration giving the total lung volume, rest half-way between a normal inspiration and expiration giving the middle capacity of the lungs, and maximum expiration leaving the residual air within the lungs. The ratios between the "chest volumes" and the lung volumes were worked out and found to be 55 for the total capacity, 37 for the middle capacity, and 19 for the residual air. The individual variations were within 10 per cent of these averages. Therefore, the chest volume multiplied by the factor thus determined gives the lung volume normal for a person of ascertained chest measurements.

The technique of determining the lung volumes and measuring the chest is fully described in Paper I. On the basis of our results there reported we have made an investigation of the lung volumes in 51 adult patients suffering from pulmonary tuberculosis. This paper is a report of our findings in 31 men. Our results in 20 women are reported in Paper III. The technique has been exactly as previously described. All the determinations were done with the patients in standing position. (1) The residual air was determined by the dilution method. As a rule, two determinations were done, and in some instances several. The lowest value was taken. (2) The vital capacity was then determined by expiration into a calibrated, easily movable Krogh spirometer. The expirations were continued until constancy was obtained. (3) The middle capacity was determined by normal breathing from the spirometer, which contained about 50 per cent oxygen, and the movements of the spirometer were recorded. When sufficient constancy appeared, the patient was asked to inspire and expire as much as possible, the vital capacity being controlled in this way. (4) If any doubt existed about the reliability of the experiments, a control was obtained by measuring the total capacity by the dilution method.

Having determined the lung volume, the chest measurements were taken in the three main positions, as previously described (Lundsgaard and Van Slyke). In a number the measurements were checked by two of us. Determinations of the movements of the diaphragm on a maximum respiration by means of x-ray (fluoroscopy) were then performed. The values were corrected (multiplied by 0.7) for parallax.

The other part of the investigation, the clinical examination of the patient, was completed within a few days after the lung and chest measurements. It consisted of (1) stethoscopic examination, (2) two x-ray plates, one taken from the front and one from the back, (3) determination of the influence of a certain amount of exercise on the pulse rate and respiration (Table I). The results are given on a chart for each case (Text-figs. 1 to 31). The different chest measurements are reported, because the relation between them gives some information about the form of chest in each particular case. The product of these measures is called chest volume, for the sake of convenience, although, of course, it is only approximately proportional to and not equal to the real chest volume. The lung capacities in the three main positions are given, and the ratios between the chest and lung volumes calculated. Besides this the ratio between the vital capacity and the middle chest volume is calculated, the normal ratio being 35 to 47. Two columns represent in diagrammatic form the calculated lung volume[6] and that actually found. The lowest (cross-hatched) part indicates the residual air, the rest is the vital capacity which is divided by a line indicating the upper limit of the middle capacity. The movements of the diaphragm are given in centimeters, and two lines indicate approximately the position of the midriff in maximum expiration and maximum inspiration. The stethoscopic and roentgenological findings are shown on four diagrams of the chest wall. The following symbols are used:

Physical Signs.—
Light lines, slight dullness.
Heavy lines, moderate dullness.
Cross-hatching, marked dullness.

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[6] The calculated lung volume means simply the chest volume multiplied by the (average) ratio for normal subjects which was established in Paper I.

Fine dots, fine râles.
Larger dots, moderate and coarse râles.
Small rings, large crackling râles.
Crosses, pleuritic rubs.
Circles, antrum formation.

There is no difference in the interpretation of horizontal and vertical lines.

X-Ray Signs.—

Lightly shaded lines, slight density of shadow.
Heavy lines, marked density.
Circles, cavity.
Dots, stippling, the larger the dots, the coarser the stippling.

There is no difference in the interpretation of horizontal and vertical lines.

A short description of each case is also given. The observations on the pulse and respiration before and after exercise are not given in the individual charts but are collected in Table I. In order to compare the findings from the different patients, the results are presented together in Text-figs. 32, 33, and 34, in the same way as the normal individuals were shown in a previous paper.[7]

It will be unnecessary to discuss each patient. We have therefore divided the patients into three groups according to the severity of the objective symptoms:[8] the incipient, the moderately advanced, and the advanced cases.

Patients with Incipient Tuberculosis.

Group I (Text-fig. 32) indicates nine patients (Nos. 1 to 9). The results in this group deviate appreciably but not greatly from the normal. The most conspicuous difference from the normal individuals is that the vital capacity[9] is moderately diminished in all but one case (No. 1). This is entirely due to an increase in the residual air. All the figures for the total capacity are within the normal limits; four below and five above the normal average. The residual air, on the other hand, is above the normal average in all but one

[7] See Text-fig. 6 of Paper I.

[8] We have followed the classification of The American Climatological Association which is based principally on Trudeau's scheme (cited in Rathbun, W. L., *Am. Rev. Tuberc.*, 1917, i, 13).

[9] See the ratio for the vital capacity on the individual charts (Text-figs. 1 to 31).

patient (No. 1). The middle capacity, which was determined in all but one patient is not far from the normal limits. The results in this group (1) serve to confirm Hutchinson's observations[10] that the vital capacity was diminished even in early tuberculosis, and (2) they show that this decrease in incipient tuberculosis is not due to a diminished total lung volume, as previously supposed, but to an increased residual air. The increased residual air is the result of an inability to expire as deeply as normally. This inability to expire is apparent in the decreased movement of the diaphragm and the decreased difference between the chest volume after total expiration and in the middle position. Whether it is mechanically caused, by stiffness of the lungs, or is due to a reflex preventing compression, we cannot tell.

Patients with Moderately Advanced and Advanced Tuberculosis.

Group II includes thirteen moderately advanced cases (Text-fig. 33, Nos. 10 to 22) and Group III nine advanced cases (Text-fig. 34, Nos. 23 to 31). The two groups can be discussed together because the differences are not great. The picture here differs materially from that found in normal individuals and in the incipient cases. The vital capacity is diminished in all the patients, in most of them very considerably (see the value of the vital ratio in Text-figs. 1 to 31; the normal is 42). The reason for this decrease is, however, principally a decrease in the total capacity, which is only within normal limits in five patients in Group II (Nos. 11, 12, 14, 16, and 19) and two patients in Group III (Nos. 26 and 28). The residual air is, in most of the patients, fairly normal. An increase in the residual air is found only in Nos. 11, 12, 18, and 19 in Group II, and Nos. 26, 27, 28, and 30 in Group III. But, as a whole, it can be said that the vital capacity in the cases in these two groups is considerably diminished, due principally to a diminished total capacity. The cause of the diminished total capacity is not principally due to an impossibility to extend the thorax, as will be seen from the figures for the chest volumes. A comparison of the figures for the chest volumes in the three positions in the individual sub-

[10] Later investigators, as mentioned, have reported the same observations.

jects shows this. The essential reason is simply that the lungs do not have so much air space as in normal individuals. It is a direct expression of one phase of the pathologic anatomic process, the proliferation. Actual cavities may presumably increase the air

TABLE I.

The Influence of Change of Position and of Exercise on Pulse and Respiration.

No. on individual diagrams.	Case No.	Resting in bed.		Standing up.		After having run up three flights of stairs.		
		Pulse.	Respirations.	Pulse.	Respirations.	Pulse.	Respirations.	Other symptoms.
Group I.								
1	4315	72	11	106	20	110	20	None.
2	3606	66	14	70	16	98	14	Headache.
3	4362	74	16	86	20	100	18	Slight palpitation and dyspnea
4	4280	70	14	80	18	102	28	" dyspnea; slight flush.
5	4197	96	16	106	18	120	18	" palpitation and dyspnea.
6	4184	72	15	88	18	102	20	" dyspnea.
7	4326	72	15	92	18	98	18	" "
9	4254	64	16	72	18	88	18	None.
Group II.								
10	4028	76	20	126	24	132	24	"
11	4148	102	22	98	22	108	28	Irregular pulse; slight dyspnea.
13	4229	78	24	100	22	106	20	Moderate dyspnea.
14	4090	68	14	112	14	120	14	Slight " slight palpitation
15	4039	74	14	96	16	114	16	None.
16	3918	76	16	104	20	104	16	Moderate dyspnea; palpitation.
17	4363	80	16	100	20	110	20	Slight palpitation and dyspnea.
18	3997	88	18	100	20	120	22	Moderate dyspnea.
19	4268	64	15	102	18	102	24	Slight "
20	4006	66	12	112	14	120	14	" " slight palpitation.
21	4076	72	18	100	18	88	20	Moderate "
22	4082	72	8*	100	10	112	10	Slight flush.
Group III.								
25	4300	100	14	116	16	120	16	Moderate dyspnea.
26	4127	72	14	100	14	116	14	Slight "
27	4317	98	20	110	24	120	28	Moderate " headache; flushes.
28	4346	70	14	98	16	104	14	None.
29	3952	76	12	136	14	126	16	Slight dyspnea; flush.
31	4130	112	20	120	17	120	30	Marked " tremors; flush.

* Verified three times.

capacity of the lungs, but none of our data bears evidence of this increase. Probably the effect of cavity formation is overcome by that of the proliferation. The difference in the residual air in incipient and advanced cases is peculiar; we shall not discuss it. Previous investigators found, as we also have found, a decrease in the vital capacity corresponding to an increase in the clinical symptoms (already shown by Hutchinson in nine patients in 1846).

We attempted to discover which of the clinical signs would correspond most closely to our findings, but have given this up. However, it seems that the stethoscopic findings, particularly the extent of the râles, have a closer relation than the x-ray shadows to the decrease in total and vital capacities. More light on this problem is highly desirable. We believe that the best way to add to present knowledge will be to follow single patients over considerable periods of time, comparing the clinical findings with the pulmometry. In Table I we have given the results of our determinations of the pulse rate and respiration before and after exercise. We think that no conclusions can be drawn from them at present. It is worth mentioning that exercise influences the rate of respiration only to a small extent, whereas the pulse rate seems to be abnormally increased. The determinations of the movements of the diaphragm show a smaller excursion than we found in normal subjects.[7] The significance of this, as far as the lung volumes are concerned, has already been mentioned. What relation it has to the pathologic process in the lungs is not yet clear.

SUMMARY.

1. The total capacity, middle capacity, and residual air have been determined in 31 adult male patients suffering from tuberculosis of the lungs.

2. The chest volumes have been determined in each case and the normal lung volumes calculated by means of the ratios worked out in a previous paper.

3. In nine patients with incipient tuberculosis, the total lung volume was found within normal limits, whereas the vital capacity was diminished as a result of an increased residual air.

The increase in the residual air was due to less complete expira-

tion, caused partly by diminished movement of the diaphragm, partly by diminished compression of the chest wall. The diminished movement of the diaphragm was, as a rule, most marked on the most affected side. Whether these decreased movements are due to a reflex or to stiffness of the lung tissue we could not determine.

The middle capacity was found practically normal.

4. In twenty-two cases of moderately advanced and advanced tuberculosis, the total lung volume was in most cases markedly decreased.

The vital capacity was substantially decreased, principally as a result of the diminished total capacity. The residual air was, as a rule, normal, although in a few cases an increase in residual air also contributed to the decrease in the vital capacity.

The middle capacity, on which we do not want to put too much stress, was normal in some patients and considerably diminished in others.

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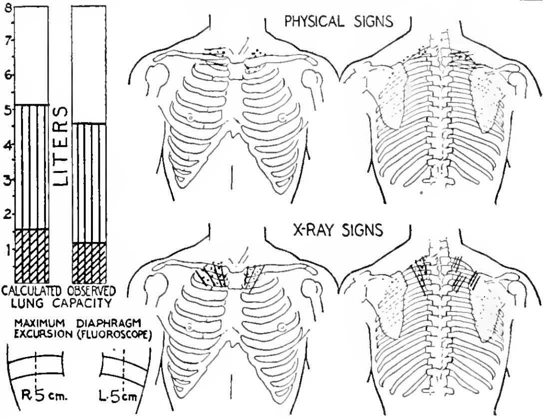
[11] For the other papers quoted see the bibliography in Paper I.

No. 1 (CASE 4315)

POSITION	CHEST DIMENSIONS: STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"	LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL
	cm.	cm.	cm	liters	liters		
REST	20.8	16.1	24.9	8.30	—	—	41.0
MAX INSP.	20.8	17.0	26.4	9.35	4.60	49.2	—
MAX EXP.	20.8	15.8	24.7	8.13	1.20	14.8	—



TEXT-FIG. 1.

No. 1 (Case 4315).—Male, elevator operator; age 26 years. Incipient; inactive. Sputum— — —, on admission, in course of treatment, and at present.

Illness began about 7 years ago with expectoration, night sweats, and pain in left chest. Moderate loss in weight; moderate dyspnea. 3 months ago slight cough, moderate expectoration, slight dyspnea, and pain at left base. His general and his lung conditions have improved satisfactorily under sanatorium treatment.

	kg.
Height 165 cm. Theoretical normal weight	62.0
Present weight	59.0
Patient's idea of normal weight	56.5
Date of highest weight 0 months ago	59.0
" " lowest " 7 " "	48.5

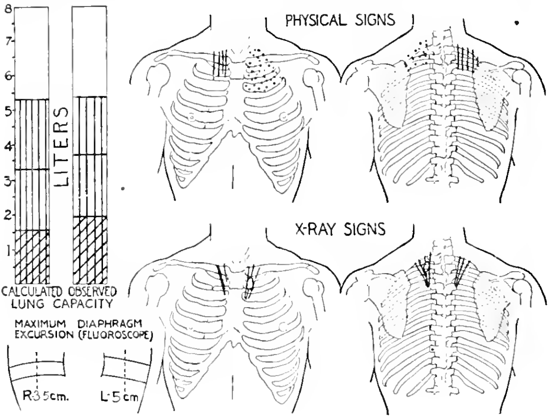
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Moderate dullness at right apex. No great change in breath sounds. Few coarse râles on cough above right clavicle, posteriorly a few clicks above spine of scapula. Fine râles on cough at left apex above clavicle and in the first interspace. Posteriorly a few fine râles on cough above the spine of the scapula.

X-Ray Signs.—April 7, 1917. Right apex and first interspace moderately infiltrated. Slight infiltration of apex and first interspace on left side. Mediastinal contents centrally placed.

No. 2 (CASE 3606)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm.	liters	liters		
REST	20.5	18.0	23.8	8.78	3.67	41.7	39.8
MAX INSP.	20.5	18.8	25.2	9.71	5.42	55.8	——
MAX EXP.	20.5	17.1	23.3	8.17	1.92	23.4	——



TEXT-FIG. 2.

No. 2 (Case 3606).—Male, butcher; age 27 years. Incipient; inactive. Sputum – ± –, on admission, in course of treatment, and at present.

Onset 30 months ago with cough. Gastric disturbances; loss of 3 kg. in weight; marked loss in strength. Slight hemoptysis 2 years ago. Under sanatorium treatment his cough has entirely disappeared; expectoration has lessened; general physical condition improved; lung signs improved.

	kg.
Height 174 cm. Theoretical normal weight	68.0
Present weight	60.0
Patient's idea of normal weight	60.0
Date of highest weight 12 months ago	65.5
" " lowest " 28 " "	55.5

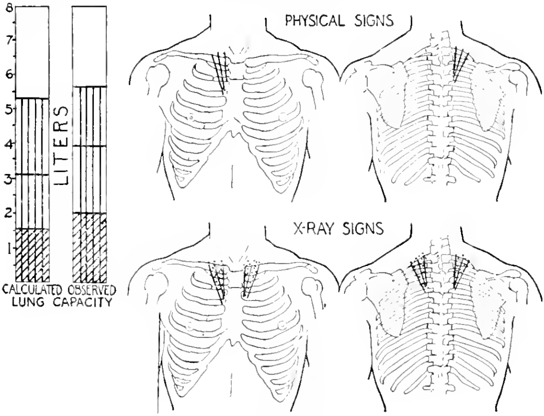
Treatment duration 22 months.

Physical Signs.—April 9, 1917. Slight dullness on percussion at right apex. Breath sounds at right apex slightly harsh. No râles. No change in percussion of left lung. Breath sounds slightly weak at apex. Fine râles on cough at apex to second rib anteriorly and to the third dorsal spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex slightly stippled and infiltrated. Left apex densely infiltrated. Chest below inner end of clavicle has a circular cavity $1\frac{1}{2}$ cm. in diameter. Mediastinal contents normal.

No. 3 (CASE 4362)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.5	17.6	24.6	8.47	4.03	47.7	45.8
MAX INSP	19.5	19.1	26.1	9.73	5.68	58.4	——
MAX EXP	19.5	17.0	24.0	7.95	2.08	26.1	——



TEXT-FIG. 3.

No. 3 (Case 4362).—Male, chauffeur; age 29 years. Incipient; inactive. Sputum + on admission.

Onset 9 months ago. Malaise and tendency to tire easily; later a few night sweats; loss of 2 kg. in weight. Doing well under sanatorium treatment, with no marked symptoms.

	kg.
Height 173 cm. Theoretical normal weight	68.5
Present weight	62.5
Patient's idea of normal weight	63.0
Date of highest weight 13 months ago	63.0
" " lowest " 4 " "	59.5

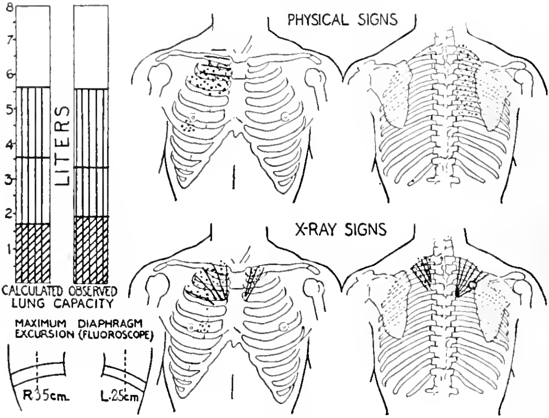
Treatment duration 1 month.

Physical Signs.—April 9, 1917. Slight dullness at right apex. No great change in breath sounds. No râles heard.

X-Ray Signs.—April 7, 1917. Right apex slight haze. Rest of lung normal. Left apex very slight haze. Rest of lung normal. Posteriorly infiltration of both apices more dense than anteriorly. Mediastinal contents normal.

No. 4 (CASE 4280)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.7	18.8	26.4	9.76	3.30	33.7	37.0
MAX INSP.	19.7	19.3	27.1	10.28	5.58	54.3	——
MAX EXP.	19.7	18.4	25.8	9.35	2.00	21.4	——



TEXT-FIG. 4.

No. 4 (Case 4280).—Male, furrier; age 27 years. Incipient; inactive. Sputum — — —, on admission, in course of treatment, and at present.

Illness began 13 months ago with pleurisy at right base, chills, and night sweats. Cessation of symptoms after 3 weeks until 6 months ago, then return of night sweats, with considerable pain in chest. Under sanatorium treatment his general condition has remained continuously good and he has had practically no symptoms.

	kg.
Height 171 cm. Theoretical normal weight	68.0
Present weight	65.0
Patient's idea of normal weight	67.0
Date of highest weight 1913	68.0
" " lowest " 7 months ago	59.5

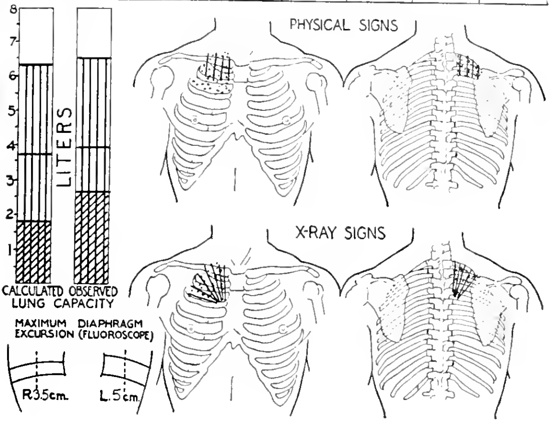
Treatment duration 4 months.

Physical Signs.—April 9, 1917. Moderate dullness at right apex to second rib. No marked change in breath sounds. Medium moist râles on cough at right apex from clavicle to third rib anteriorly, and from the apex to an inch above the angle of the scapula posteriorly. A few medium râles on cough below the right nipple.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. In the fourth interspace a small spot of stippling 3 cm. in diameter. A cavity posteriorly in the third interspace 2½ cm. in diameter. Left apex slightly stippled. Mediastinal contents normal.

No. 5 (CASE 4197)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.3	18.8	25.2	10.1	3.95	39.1	38.0
MAX INSP.	21.3	19.9	27.2	11.5	6.55	57.0	——
MAX EXP.	21.3	18.0	25.0	9.6	2.70	28.1	——



TEXT-FIG. 5.

No. 5 (Case 4197).—Male, glove cutter; age 19 years. Incipient; inactive. Sputum − + +, on admission, in course of treatment, and at present.

Present illness began 8 months ago with moderate hemoptysis. Later moderate cough with profuse expectoration. Occasional night sweats. He has been in good general condition during his stay in the sanatorium; still has a slight cough and slight expectoration. His lung condition seems unchanged.

	kg.
Height 175 cm. Theoretical normal weight	67.5
Present weight	71.5
Patient's idea of normal weight	68.0
Date of highest weight 4 months ago	72.5
" " lowest " 19 " "	66.0

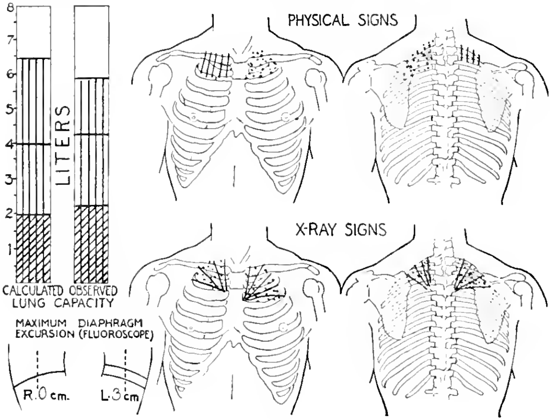
Treatment duration 6 months.

Physical Signs.—April 9, 1917. Slight dullness at right apex to second rib anteriorly and third spine posteriorly. Breath sounds slightly harsh in the same area. Fine râles on cough from the apex to the third rib anteriorly and to the third spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Mediastinal contents normal.

No. 6 (CASE 4184)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	22.4	16.9	28.1	10.7	4.30	40.0	34.1
MAX INSP.	22.4	17.9	29.1	11.7	5.85	50.0	——
MAX EXP.	22.4	16.7	27.8	10.4	2.20	21.1	——



TEXT-FIG. 6.

No. 6 (Case 4184).—Male, machinist; age 20 years. Incipient; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Present illness began 10 months ago with malaise, slight morning cough, and expectoration; frequent night sweats. Pain in upper part of right lung. Streaked sputum occasionally for the first 3 months. His general condition has been good under sanatorium treatment; still has slight cough with moderate expectoration, occasionally blood-streaked. His lung signs have slightly increased.

	kg.
Height 178 cm. Theoretical normal weight	69.5
Present weight	75.0
Patient's idea of normal weight	71.5
Date of highest weight 4 months ago	76.0
" " lowest " 7 " "	69.0

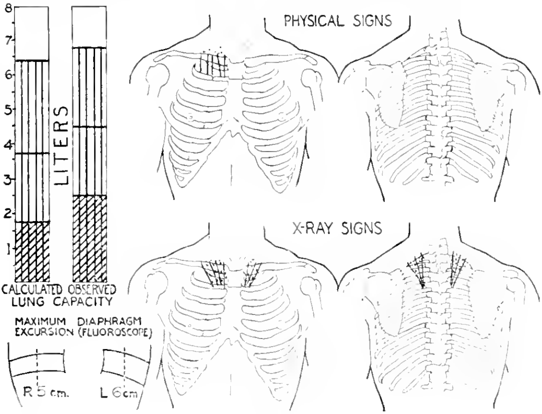
Treatment duration 6 months.

Physical Signs.—April 9, 1917. Slight dullness at right apex to second rib anteriorly and to the second spine posteriorly. Breath sounds slightly harsh in this area. Medium moist râles on cough at left apex to the second rib anteriorly and to the third dorsal spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first interspace slightly infiltrated. Left apex and first and second interspaces slightly stippled and infiltrated. Mediastinal contents normal.

No. 7 (CASE 4326)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 × LUNG CAP / CHEST VOL	RATIO 100 × VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm	liters	liters		
REST	20.7	19.0	26.1	10.25	4.51	44.0	41.9
MAX INSP.	20.7	20.8	27.3	11.75	6.83	58.1	——
MAX EXP.	20.7	18.4	25.5	9.71	2.53	24.5	——



TEXT-FIG. 7.

No. 7 (Case 4326).—Male, butcher; age 38 years. Incipient; inactive. Sputum — — —, on admission, in course of treatment, and at present.

Onset 26 months ago with malaise and tendency to tire readily. 6 months ago fever, chills, slight dyspnea, slight cough, and expectoration. His general condition has been greatly improved since admission to the hospital and his lung condition markedly bettered.

	kg.
Height 173 cm. Theoretical normal weight	71.5
Present weight	68.0
Patient's idea of normal weight	59.0
Date of highest weight 1 month ago	68.5
" " lowest " 4 months "	59 0

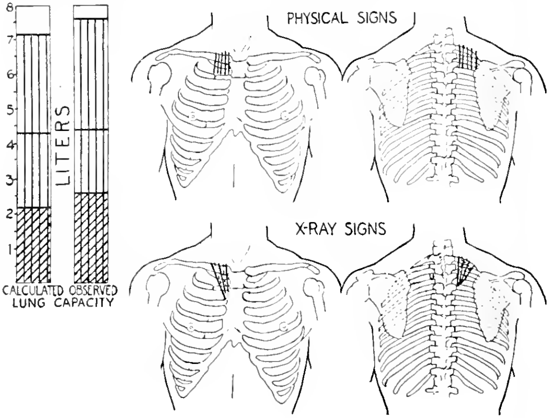
Treatment duration 2 months.

Physical Signs.—April 9, 1917. Slight dullness at right apex, especially at inner end of first interspace. Breath sounds slightly increased at left apex, both anteriorly and posteriorly. Very few fine râles on cough at right apex above the clavicle.

X-Ray Signs.—April 7, 1917. Right apex moderately densely spotted and striated. Left apex moderately densely spotted and striated. Mediastinal contents normal.

No. 8 (CASE 3651)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm	cm.	cm	liters	liters		
REST	23.0	19.0	26.5	11.60	4.37	37.7	44.0
MAX INSP	23.0	20.0	28.5	13.10	7.67	58.2	——
MAX EXP.	23.0	18.0	26.0	10.60	2.57	24.2	——



TEXT-FIG. 8.

No. 8 (Case 3651).—Male, machinist; age 31 years. Incipient; inactive. Sputum + ± −, on admission, in course of treatment, and at present.

Onset 6 years, 8 months ago with cough and night sweats. Loss of 4.5 kg. in weight. Frequent small hemoptyses. Expectoration slight. Under sanatorium treatment he has remained in very good general condition with good improvement in lung condition.

	kg.
Height 183 cm. Theoretical normal weight	77.0
Present weight	75.0
Patient's idea of normal weight	74.0
Date of highest weight 8 years ago	79.5
" " lowest " 7 months "	74.0

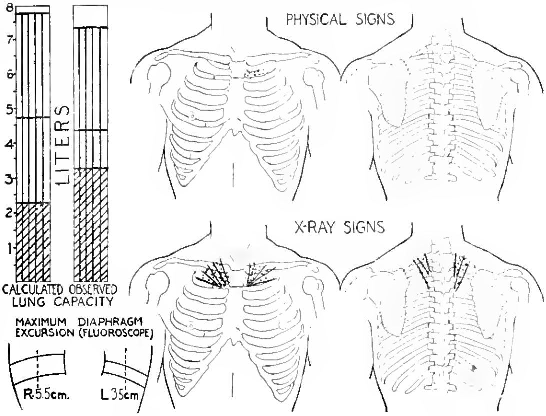
Treatment duration 22 months.

Physical Signs.—April 9, 1917. Percussion resonance of right apex slightly impaired. Breath sounds slightly increased at right apex. No râles heard, before or after cough. The patient had a small hemorrhage of 5 cc. 21 hours after the measurements were taken, caused by slipping on ice.

X-Ray Signs.—Right apex quite densely spotted and striated. Mediastinal contents normal.

No. 9 (CASE 4254)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm	cm.	cm.	liters	liters		
REST	20.0	21.1	29.8	12.60	4.27	33.9	32.5
MAX INSP.	20.0	22.8	31.7	14.50	7.39	50.9	——
MAX EXP.	20.0	20.3	29.4	11.90	3.27	27.5	——



TEXT-FIG. 9.

No. 9 (Case 4254).—Male, carpenter; age 38 years. Incipient; active. Sputum — ± —, on admission, in course of treatment, and at present.

Onset 8 months ago with cough. Expectoration slight at first, later moderate. Headaches; slight dyspnea. Loss of 3 kg. in weight. His lung condition has improved under sanatorium treatment and his general physical condition has been excellent.

	kg.
Height 170 cm. Theoretical normal weight	69.5
Present weight	87.5
Patient's idea of normal weight	80.5
Date of highest weight 0 months ago	87.5
" " lowest " 8 " "	76.5

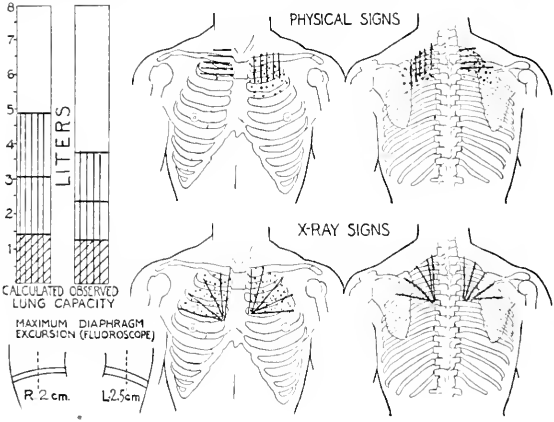
Treatment duration 5 months.

Physical Signs.—April 9, 1917. No great percussion changes. No marked changes in respiratory sounds. Few fine râles on cough at the inner end of the first interspace on the left side.

X-Ray Signs.—April 7, 1917. Right apex and first interspace densely infiltrated. Left apex and first interspace slightly infiltrated. Mediastinal contents normal.

No. 10 (CASE 4028)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.0	16.9	25.5	8.18	2.35	28.8	29.4
MAX INSP.	19.0	17.8	26.6	9.0	3.70	41.3	——
MAX EXP.	19.0	15.7	25.3	7.56	1.30	17.2	——



TEXT-FIG. 10.

No. 10 (Case 4028).—Male, student; age 17 years. Moderately advanced; active. Sputum − + +, on admission, in course of treatment, and at present.

Onset 13 months ago with a cold. Cough severe; expectoration slight. Moderate dyspnea. Occasional chills. 2 weeks after onset a profuse hemoptysis. Loss of 2.7 kg. in weight. His symptoms remain about the same under sanatorium treatment. Physical signs have increased. General condition remains fair.

	kg.
Height 168 cm. Theoretical normal weight	57.5
Present weight	61.0
Patient's idea of normal weight	59.0
Date of highest weight 0 months ago	61.0
" " lowest " 7 " "	48.0

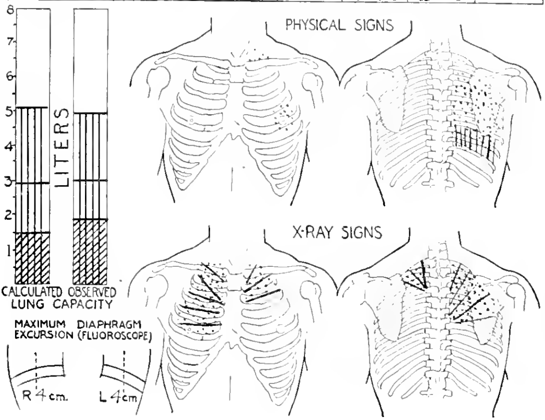
Treatment duration 11 months.

Physical Signs.—April 9, 1917. Moderate dullness at right apex to second rib anteriorly and third spine posteriorly. Slight dullness at left apex to second rib anteriorly and third spine posteriorly. Breath sounds moderately harsh at right upper thorax. Breath sounds feeble at left upper thorax. Râles on cough, fine and medium, at right apex to fourth spine posteriorly. Medium râles on cough at left apex to third rib anteriorly and fourth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first, second, and third interspaces moderately stippled. Left apex and first, second, and third interspaces moderately stippled. Mediastinal contents normal.

No.11(CASE 4148)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.2	17.2	24.1	7.92	3.05	38.5	37.7
MAX INSP	19.2	18.6	25.9	9.25	4.90	53.0	——
MAX EXP.	19.2	16.8	23.9	7.72	1.90	24.6	——



Text-Fig. 11.

No. 11 (Case 4148).—Male, freight house clerk; age 23 years. Moderately advanced; inactive. Sputum + ± −, on admission, in course of treatment, and at present.

Onset 18 months ago with hemoptysis. Cough moderate; expectoration slight. Few night sweats. Loss of 4.5 kg. in weight. During his sanatorium stay he has remained in good general condition. Lung condition slightly improved.

	kg.
Height 175 cm. Theoretical normal weight	69.0
Present weight	59.5
Patient's idea of normal weight	56.0
Date of highest weight 1 month ago	60.5
" " lowest " 7 months "	50.5

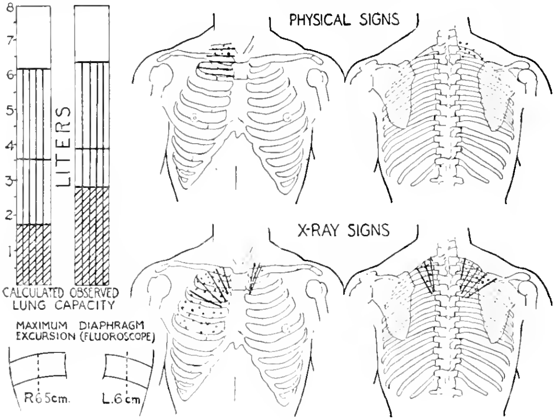
Treatment duration 7 months.

Physical Signs.—April 9, 1917. Slight dullness at right base posteriorly. No great change in breath sounds. Fine moist râles on cough at left apex above the clavicle. Fine moist râles on cough at the left anterior third and fourth interspaces in the region of the nipple. Fine moist râles on cough posteriorly on the right side from the spine of the scapula above to the base

X-Ray Signs.—April 7, 1917. Right, fine stipplings from the apex to the fourth interspace. Left, moderate stippling from the apex to the second interspace. Mediastinal contents slightly to the right.

No. 12 (CASE 3990)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.0	20.0	24.0	9.60	3.89	40.5	37.5
MAX INSP.	20.0	22.0	25.5	11.20	6.34	56.7	——
MAX EXP.	20.0	19.5	23.5	9.20	2.74	29.8	——



TEXT-FIG. 12.

No. 12 (Case 3990).—Male, machinist; age 25 years. Moderately advanced; inactive. Sputum − − −, on admission, in course of treatment, and at present.

Onset 30 months ago with hemoptysis. Loss of 4 kg. in weight. Pain in chest slight. Slight dyspnea. Slight expectoration. During his hospital stay he has remained in fair general condition with moderate improvement in lung condition.

	kg.
Height 170 cm. Theoretical normal weight	65.5
Present weight	65.5
Patient's idea of normal weight	60.5
Date of highest weight 10 months ago	68.0
" " lowest " 2 years "	58.5

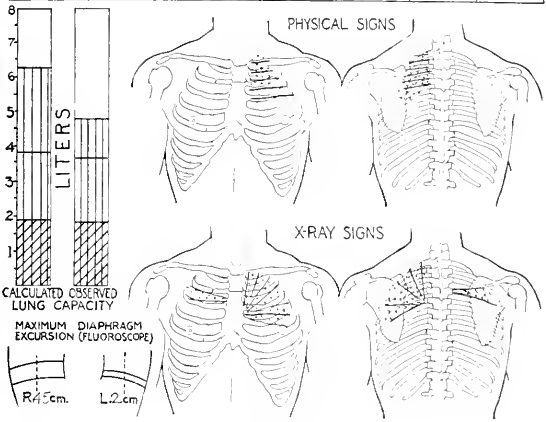
Treatment duration 12 months.

Physical Signs.—April 9, 1917. Dullness at upper part of right lung anteriorly to the second rib. Increased breath sounds at upper part of right lung anteriorly to the second rib and posteriorly to the spine of the scapula. Medium moist râles on cough at the right apex, above the clavicle anteriorly, and to the spine of the scapula posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces quite densely spotted and striated. The third and fourth interspaces show very fine spottings. Left apex slightly spotted; rest of lung normal. Mediastinal contents normal.

No. 13 (CASE 4229)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.0	18.8	26.1	10.30	3.75	36.4	28.1
MAX INSP.	21.0	19.7	27.4	11.35	4.70	41.4	——
MAX EXP.	21.0	18.4	25.8	10.00	1.80	18.0	——



TEXT-FIG. 13.

No. 13 (*Case 4229*).—Male, music teacher; age 42 years. Moderately advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 12 months ago with heavy cold. Cough moderate; expectoration profuse after 2 weeks. Dyspnea moderate. Loss of 2.7 kg. in weight. 2 months after onset profuse hemoptysis. Pain in left side. Under treatment in the hospital he has improved in general condition; cough and expectoration moderate. The physical signs remain about the same.

	kg.
Height 170 cm. Theoretical normal weight	70.0
Present weight	65.0
Patient's idea of normal weight	61.0
Date of highest weight 2 months ago	65.5
" " lowest " 5 " "	58.5

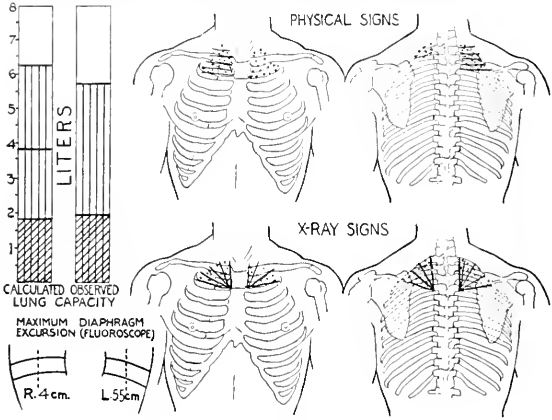
Treatment duration 5 months.

Physical Signs.—April 9, 1917. Moderate dullness on percussion over the left apex and from the apex to the third rib anteriorly and to the fourth spine posteriorly. Breath sounds moderately harsh in this area. Râles on coughing, fine and medium, from apex to the second rib anteriorly and to the fifth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right lung, second interspace slightly stippled. Left upper lobe moderately densely spotted and stippled. Mediastinal contents slightly to the left above.

No. 14 (CASE 4090)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm	cm	cm.	liters	liters		
REST	20.5	18.7	27.0	10.4	—	—	32.7
MAX INSP.	20.5	20.0	27.9	11.4	5.7	50.0	—
MAX EXP.	20.5	17.8	26.2	9.6	2.0	20.9	—



TEXT-FIG. 14.

No. 14 (Case 4090).—Male, lithographer; age 32 years. Moderately advanced; inactive. Sputum + ± +, on admission, in course of treatment, and at present.

Present illness began 13 months ago with neurasthenic symptoms, insomnia, etc. 2 months later slight cough with scanty expectoration; no other symptoms. Under sanatorium treatment his cough has lessened. Has complained of insomnia. General physical condition has bettered. His lung signs have improved slightly.

	kg.
Height 177 cm. Theoretical normal weight	74.5
Present weight	76.0
Patient's idea of normal weight	72.5
Date of highest weight 8 years ago	85.0
" " lowest " 7 months "	69.5

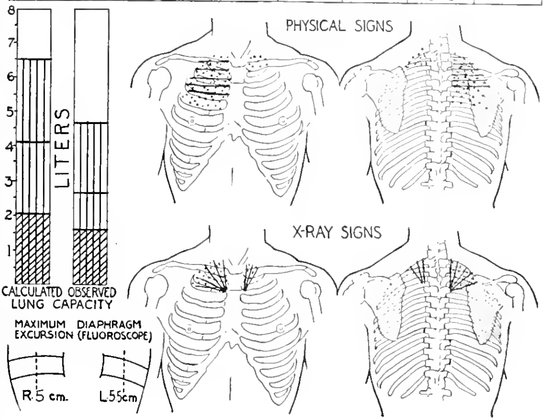
Treatment duration 9 months.

Physical Signs.—April 9, 1917. Moderate dullness at right apex to second rib anteriorly and to third spine posteriorly. Moderately harsh breathing in this area. Breath sounds slightly feeble at left apex. Fine râles at right apex to second rib anteriorly and to third spine posteriorly. Fine râles on cough at left apex to second rib anteriorly and to second spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Left apex and first interspace similarly infiltrated. Mediastinal contents normal

No. 15 (CASE 4039)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	21.5	19.6	26.5	11.2	2.65	23.7	26.9
MAX INSP	21.5	20.0	27.4	11.8	4.6	39.0	——
MAX EXP	21.5	18.9	26.1	10.6	1.6	15.0	——



TEXT-FIG. 15.

No. 15 (*Case 4039*).—Male, clothing cutter; age 28 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 6 years ago with cold. Persistent cough. Hemoptysis slight 6 months after onset. 2 years ago series of severe hemoptyses. Artificial pneumothorax. Has felt well and is near his normal weight since recovery after hemoptyses. Under sanatorium treatment his symptoms have remained slight; very little cough, slight expectoration. His physical condition has been excellent.

		kg.
Height 175 cm. Theoretical normal weight		70.5
Present weight		72.0
Patient's idea of normal weight		72.5
Date of highest weight	4 years ago	77.0
" " lowest "	28 months "	54.5

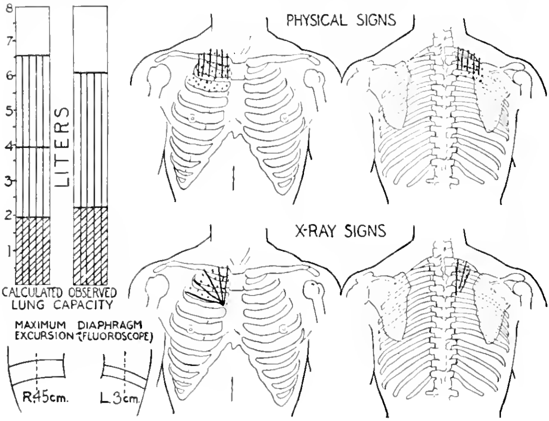
Treatment duration 10 months.

Physical Signs.—April 9, 1917. Moderate dullness on percussion at right apex to the third rib anteriorly and to the fourth spine posteriorly. Breath sounds moderately harsh. Râles on cough, fine and medium, at apex to the fourth rib anteriorly and to the sixth spine posteriorly. Breath sounds slightly feeble at left apex. Medium moist râles on cough at apex to second rib anteriorly and to the second spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first interspace densely infiltrated. Left apex slightly infiltrated. Mediastinal contents normal.

No. 16 (CASE 3918)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.4	18.1	29.4	10.75	—	—	34.8
MAX INSP.	20.4	19.1	30.8	11.93	6.10	51.3	—
MAX EXP.	20.4	17.6	28.9	10.38	2.25	21.6	—



TEXT-FIG. 16.

No. 16 (Case 3918).—Male, factory inspector; age 27 years. Moderately advanced; inactive. Sputum + + −, on admission, in course of treatment, and at present.

Onset 19 months ago with malaise, loss in strength; 2 months later fever and cough. Sputum occasionally blood-streaked. Loss of 3 kg. in weight. Occasional night sweats. Dyspnea slight. Under sanatorium treatment he has remained in fair general condition. Complications of larynx and rectal fistula have arisen. Slight hemoptysis frequent. Symptoms and lung condition remain the same.

	kg.
Height 175 cm. Theoretical normal weight	70.0
Present weight	71.0
Patient's idea of normal weight	70.0
Date of highest weight 0 months ago	71.0
" " lowest " 8 " "	63.5

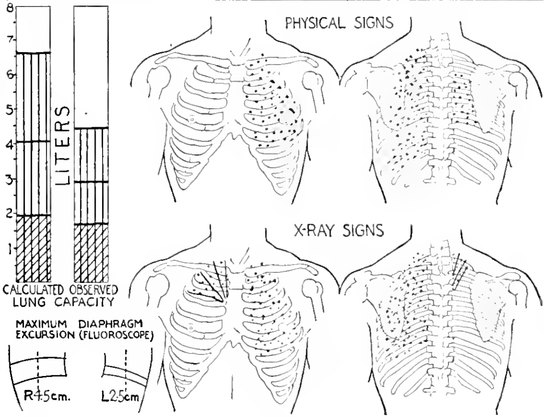
Treatment duration 13 months.

Physical Signs.—April 9, 1917. Slightly dull percussion note at right apex to second rib anteriorly and to third spine posteriorly. Breath sounds moderately harsh in the same area. Fine and medium râles on cough at right apex to the third rib anteriorly and to the fourth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right upper lobe moderately densely infiltrated to the third rib. Mediastinal contents normal.

No. 17 (CASE 4363)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	20.0	19.7	28.0	11.04	2.9	26.3	24.9
MAX INSP.	20.0	20.7	29.1	12.06	4.45	36.9	——
MAX EXP.	20.0	18.9	27.0	10.20	1.7	16.6	——



TEXT-FIG. 17.

No. 17 (Case 4363).—Male, sign writer; age 29 years. Moderately advanced; active. Sputum + on admission.

Present illness began 25 months ago with malaise and tendency to tire easily. Gastric symptoms. Loss of 4.5 kg. in weight. Later moderate cough with scanty expectoration. His physical condition remains good under sanatorium treatment; symptoms about the same. Lung condition about the same.

	kg.
Height 174 cm. Theoretical normal weight	70.0
Present weight	69.0
Patient's idea of normal weight	70.0
Date of highest weight 19 months ago	75.5
" " lowest " 17 " "	60.0

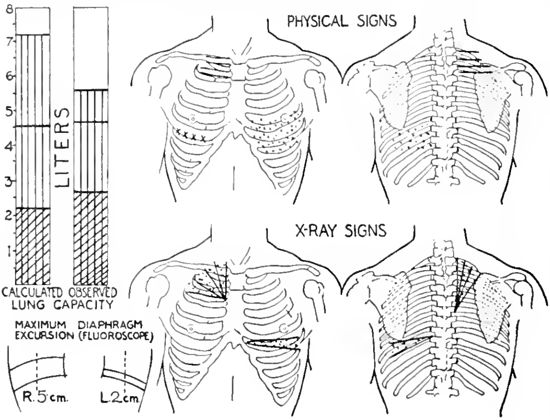
Treatment duration 1 month.

Physical Signs.—April 7, 1917. No marked percussion changes. Increased breath sounds at right apex posteriorly. Fine râles on cough posteriorly from spine to angle of scapula. Medium and coarse râles, on cough, in left lung anteriorly and posteriorly from apex to base.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces slightly infiltrated. Rest of lung normal. Entire left lung slightly infiltrated, with fine spotting. Mediastinal contents completely to the left. Right lung area large.

No. 18 (CASE 3997)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	22.0	19.1	28.9	12.2	4.62	37.7	24.2
MAX INSP.	22.0	19.9	29.9	13.1	5.57	42.5	——
MAX EXP.	22.0	18.3	28.6	11.5	2.62	22.8	——



TEXT-FIG. 18.

No. 18 (Case 3997).—Male, machinist; age 27 years. Moderately advanced; inactive. Sputum − ± +, on admission, in course of treatment, and at present.

Onset 16 months ago with moderate hemoptysis; later slight cough, occasional night sweat, and loss of 2 kg. in weight. Has felt well during his entire stay in the hospital.

	kg.
Height 174 cm. Theoretical normal weight	70.0
Present weight	76.5
Patient's idea of normal weight	66.0
Date of highest weight 6 months ago	79.0
" " lowest " ? " "	70.0

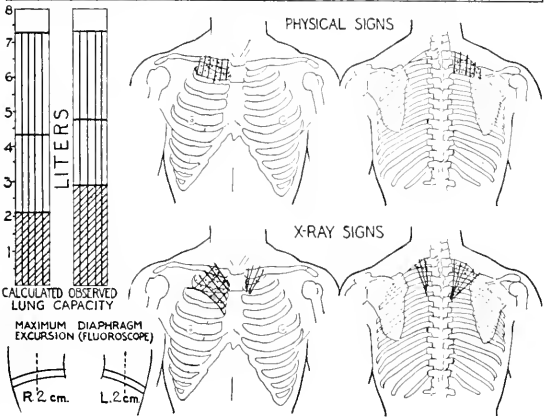
Treatment duration 12 months.

Physical Signs.—April 9, 1917. Moderate dullness at right apex, anteriorly to the second rib, posteriorly to the spine of the scapula. Breath sounds slightly increased at the left base. Fine moist râles on cough at left base, anteriorly below the fourth rib, posteriorly below a point midway between the spine and the angle of the scapula. Friction rubs at right base anteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces slightly stippled and striated. Left, fifth interspace moderately spotted and striated. Mediastinal contents normal.

No. 19 (CASE 4268)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.0	20.0	29.7	11.9	4.78	40.2	38.2
MAX INSP.	20.0	21.5	31.2	13.4	7.38	55.0	——
MAX EXP.	20.0	19.5	28.6	11.1	2.93	26.4	——



TEXT-FIG. 19.

No. 19 (Case 4268).—Male, teamster; age 29 years. Moderately advanced; inactive. Sputum + ± −, on admission, in course of treatment, and at present.

Present illness began 14 months ago after an attack of supposed influenza. Malaise; weakness; loss of 6.8 kg. in weight; 5 months after onset afternoon fever; occasional chills. Shortly after began to cough; expectoration profuse. Slight pain in left base. Under sanatorium treatment his symptoms have largely disappeared, his general condition is excellent, and the lung condition is apparently greatly improved.

	kg.
Height 186 cm. Theoretical normal weight	78.0
Present weight	87.0
Patient's idea of normal weight	84.0
Date of highest weight 3 months ago	92.0
" " lowest " 10 " "	79.5

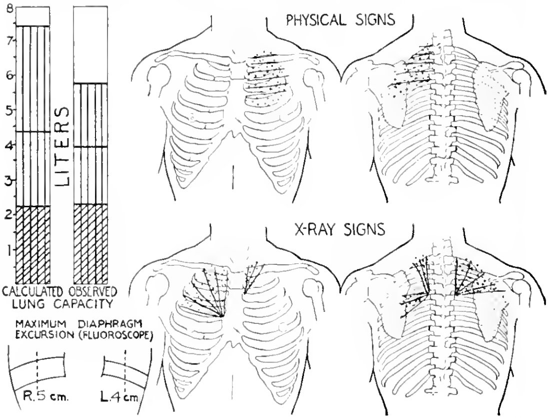
Treatment duration 5 months.

Physical Signs.—April 9, 1917. Slight dullness on percussion at right apex to the second rib anteriorly and the third dorsal spine posteriorly. Breath sounds slightly harsh. Fine râles on cough at apex to second rib anteriorly and to third spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces densely infiltrated. The lower edge of the infiltration is sharply limited from healthy lung below. Left apex infiltrated. Mediastinal contents normal.

No. 20 (CASE 4006)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 x LUNG CAP / CHEST VOL.	RATIO 100 x VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm	liters	liters		
REST	21.2	19.8	28.5	11.95	3.90	32.5	29.3
MAX INSP.	21.2	21.1	30.2	13.55	5.80	42.7	——
MAX EXP.	21.2	19.6	28.2	11.70	2.30	19.7	——



TEXT-FIG. 20.

No. 20 (Case 4006).—Male, conductor on elevated railroad; age 33 years. Moderately advanced; inactive. Sputum — — —, on admission, in course of treatment, and at present.

Present illness began 27 months ago with bronchitis. Severe cough for 2 months. Fever; night sweats; loss of 5.4 kg. in weight. Moderate weakness. Hemoptysis of moderate amount 6 months after onset. Pain at left base. Under sanatorium treatment his symptoms remain about the same except improvement in strength. No fever since onset. His general physical condition remains excellent.

	kg.
Height 166 cm. Theoretical normal weight	64.5
Present weight	77.0
Patient's idea of normal weight	84.0
Date of highest weight 4 years ago	84.0
" " lowest " 8 months "	75.0

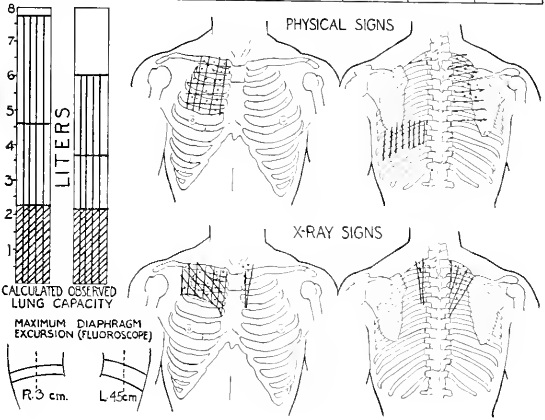
Treatment duration 11 months.

Physical Signs.—April 9, 1917. No change in percussion over right lung. Breath sounds slightly harsh at right apex. Moderate dullness at left apex to third rib anteriorly and to fourth spine posteriorly. Breath sounds feeble in the same area. Fine and medium râles on cough at left apex to fourth rib anteriorly and to fifth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first three interspaces moderately densely striated and spotted. Left apex slight spotting. Mediastinal contents normal

No. 21(CASE 4076)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.5	19.5	29.7	12.4	3.70	29.8	31.4
MAX INSP.	21.5	20.7	31.5	14.1	6.10	43.2	——
MAX EXP.	21.5	19.0	29.4	12.0	2.20	18.8	——



TEXT-FIG. 21.

No. 21 (Case 4076).—Male, farmhand; age 27 years. Moderately advanced; inactive. Sputum — — —, on admission, in course of treatment, and at present.

Onset 34 months ago with fever; loss of 24.5 kg. in weight in the first 6 months. Chills; night sweats; cough and expectoration slight. Slight hemoptysis. General condition during his hospital stay has been excellent; lung condition improved.

	kg.
Height 183 cm. Theoretical normal weight	77.0
Present weight	89.0
Patient's idea of normal weight	86.0
Date of highest weight 12 months ago	97.0
" " lowest " 34 " "	61.5

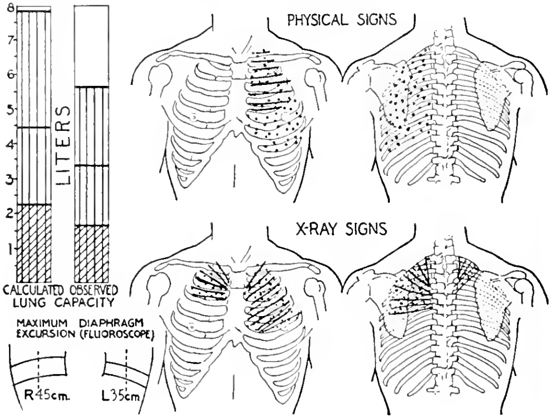
Treatment duration 10 months.

Physical Signs.—April 9, 1917. Marked dullness of right lung anteriorly to the fourth rib, posteriorly moderate dullness from apex to spine of the scapula. Slight dullness at the left base below angle of scapula. No great change in breath sounds. Medium moist râles on cough at right apex to the third rib. Scattered fine moist râles on cough at right apex posteriorly to midway between the spine and angle of the scapula.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. The lower border of this infiltration has a sharp convex line, convexity upward. Left apex moderately densely infiltrated. Mediastinal contents normal.

No. 22 (CASE 4082)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	23.8	19.0	26.7	12.08	3.34	27.6	32.7
MAX INSP.	23.8	20.8	28.8	14.25	5.59	39.3	——
MAX EXP.	23.8	18.8	25.9	11.06	1.64	14.1	——



TEXT-FIG. 22.

No. 22 (Case 4082).—Male, butcher; age 22 years. Moderately advanced; active. Sputum + ± +, on admission, in course of treatment, and at present.

Onset 13 months ago with cold. Cough and expectoration moderate; slight pain in left side; slight dyspnea. During his stay in the sanatorium his general condition has remained good and his lung condition is apparently much improved.

	kg.
Height 181 cm. Theoretical normal weight	72.5
Present weight	74.5
Patient's idea of normal weight	76.0
Date of highest weight 22 months ago	78.0
" " lowest " 8 " "	73.0

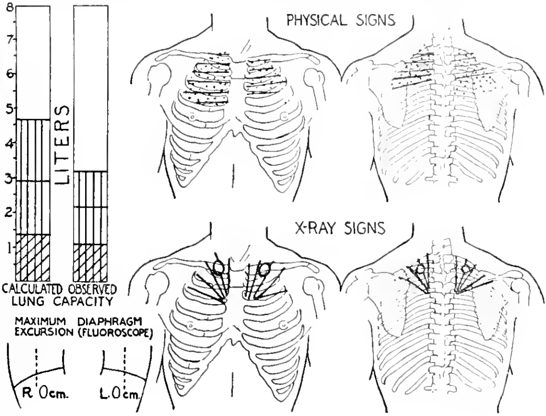
Treatment duration 10 months.

Physical Signs.—April 9, 1917. Dullness of upper part of left lung anteriorly to the fourth rib. No marked change in breath sounds. Coarse râles on cough in left lung both anteriorly and posteriorly to the base.

X-Ray Signs.—April 7, 1917. Right apex moderately densely infiltrated. First and second interspaces slightly stippled. Left apex and first and second interspaces very densely infiltrated. Third and fourth interspaces moderately spotted. Mediastinal contents to the left. Right lung area greatly increased.

No. 23 (CASE 4911)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 × LUNG CAP / CHEST VOL	RATIO 100 × VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm	liters	liters		
REST	18.9	17.4	23.6	7.8	2.1	26.9	27.0
MAX INSP.	18.9	18.0	24.4	8.3	3.2	38.6	——
MAX EXP.	18.9	17.1	23.3	7.5	1.1	14.7	——



TEXT-FIG. 23.

No. 23 (*Case 4911*).—Male, factory worker; age 19 years. Advanced; inactive. Sputum +++, on admission, in course of treatment, and at present.

Onset 17 months ago with symptoms of grippe; began to cough slightly, although weight was not lost. Diagnosis made by sputum examination. Improved rapidly under sanatorium treatment and gained weight. Cough and expectoration markedly diminished.

	kg.
Height 163 cm. Theoretical normal weight	57.0
Present weight	53.0
Patient's idea of normal weight	46.0
Date of highest weight 5 months ago	54.5
" " lowest " 11 " "	45.3

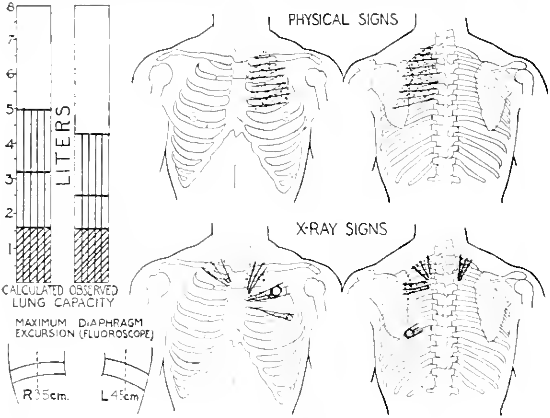
Treatment duration 11 months.

Physical Signs.—April 9, 1917. Right, moderate dullness, very harsh breathing. Numerous râles on cough to top of fourth rib anteriorly, and sixth spine posteriorly. Left, moderate dullness, harsh breathing. Râles on cough to third rib anteriorly, and fourth spine posteriorly. Signs of cavity on both sides anteriorly.

X-Ray Signs.—April 7, 1917. Right apex, cavity 4 cm. in diameter. First and second interspaces densely striated. Left apex, cavity 6 cm. in diameter. First and second interspaces densely infiltrated. Mediastinal contents normally placed.

No. 24 (CASE 3334)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm	liters	liters		
REST	19.5	17.8	24.7	8.6	2.45	28.5	30.3
MAX INSP	19.5	18.3	25.2	9.0	4.20	46.7	——
MAX EXP	19.5	17.6	24.4	8.4	1.56	18.5	——



TEXT-FIG. 24.

No. 24 (Case 3334).—Male, student; age 16 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 44 months ago with an acute cold, cough, and fever, soon followed by night sweats. On admission 32 months ago improved rapidly in general condition and weight, and is now with minimum cough and expectoration and in excellent general condition.

	kg.
Height 165 cm. Theoretical normal weight..........	59.0
Present weight..........	63.0
Patient's idea of normal weight..........	59.0
Date of highest weight 29 months ago..........	63.5
" " lowest " 3 years "	52.0

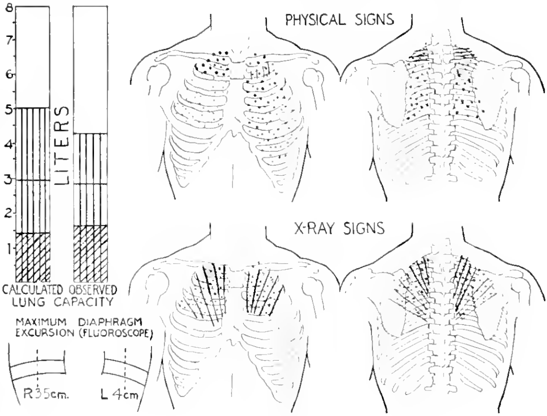
Treatment duration 32 months.

Physical Signs.—April 9, 1917. Right lung clear. Left, moderate dullness, slight harsh breathing, and numerous fine râles on cough anteriorly to fourth rib and posteriorly to sixth spine.

X-Ray Signs.—April 7, 1917. Right apex very slightly stippled Left apex densely stippled and striated. Second interspace densely infiltrated and stippled, with cavity 3 by 2 cm. In the fourth inter-pace is a very small cavity the size of a bean. Mediastinal contents normally placed.

No. 25 (Case 4300)

Position	Chest dimensions				Lung capacity	Ratio 100 × Lung cap / Chest vol	Ratio 100 × Vital cap / Chest vol
	Sternum	Ant.-post.	Transverse	"Chest volume"			
	cm	cm	cm	liters	liters		
Rest	19.2	17.0	24.5	8.0	2.86	35.8	33.7
Max insp.	19.2	18.2	26.0	9.1	4.31	47.4	——
Max exp.	19.2	16.3	24.3	7.6	1.61	21.2	——



TEXT-FIG. 25.

No. 25 (Case 4300).—Male, machinist; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 17 months ago with malaise, weakness, and loss of 9 kg. in weight. Later severe cough, moderate expectoration, and night sweats. Fever, 100–101° F. During his stay in the sanatorium the fever has subsided and cough improved; no change otherwise.

	kg.
Height 173 cm. Theoretical normal weight	66.5
Present weight	52.0
Patient's idea of normal weight	58.0
Date of highest weight 21 months ago	59.0
" " lowest " 10 " "	47.5

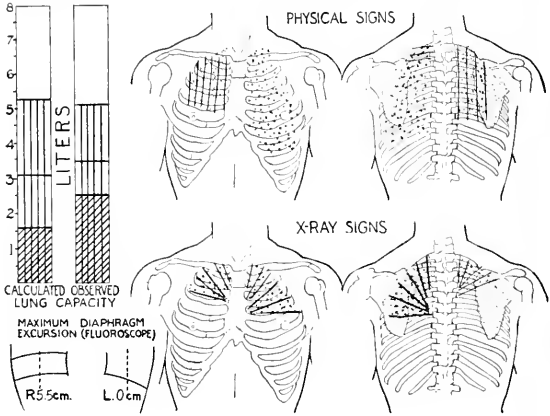
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Anteriorly slight dullness in the first interspace on the left side Dullness at both apices posteriorly. The breath sounds are increased at the right apex anteriorly and posteriorly. Coarse râles on cough at right apex to the second rib; below this fine râles to the fifth rib. Posteriorly coarse râles at upper part of right lung to the angle of the scapula. Coarse râles on cough in left lung to the base anteriorly and to the angle of the scapula posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first interspace densely infiltrated. Second and third interspaces slightly infiltrated and spotted. Rest of lung normal. Left apex and first, second, and third interspaces densely infiltrated and spotted. Mediastinal contents centrally placed. Trachea a little to the right.

No. 26 (CASE 4127)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	18.6	17.0	27.4	8.66	3.45	39.7	30.1
MAX INSP.	18.6	17.9	29.1	9.67	5.10	52.8	——
MAX EXP.	18.6	16.4	27.0	8.23	2.50	30.4	——



TEXT-FIG. 26.

No. 26 (*Case 4127*).—Male, customs inspector; age 24 years. Advanced; active. Sputum + ± −, on admission, in course of treatment, and at present.

Onset 37 months ago with slight cough and expectoration. Loss of 3 kg. in weight. Sputum blood-streaked. His general condition during his hospital stay has remained good. He has had several slight hemoptyses. His lung condition apparently is unchanged.

	kg.
Height 168 cm. Theoretical normal weight	63.0
Present weight	61.0
Patient's idea of normal weight	60.0
Date of highest weight 12 months ago	65.5
" " lowest " 18 " "	56.5

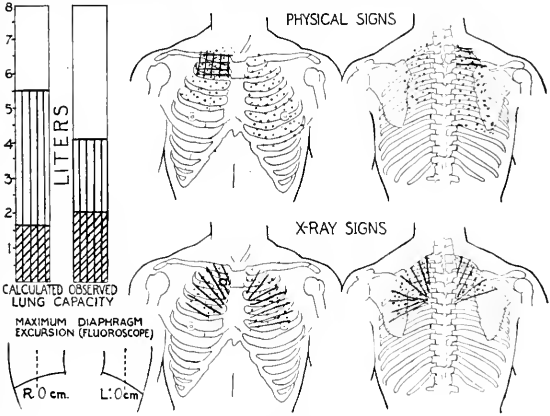
Treatment duration 8 months.

Physical Signs.—April 9, 1917. Slight dullness over right lung, anteriorly to the fourth rib and posteriorly to the angle of the scapula. Dullness at left apex anteriorly. Harsh breath sounds at right apex. Slightly increased breath sounds at left apex posteriorly. Fine moist râles on cough at upper part of right lung to the third rib anteriorly and to the angle of the scapula posteriorly. Medium moist râles on cough in left lung to the base, both anteriorly and posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left apex and first, second, and third interspaces quite densely stippled and infiltrated. Mediastinal contents are entirely to the left. Right lung area much increased.

No. 27 (CASE 4317)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.0	17.0	27.2	9.25	—	—	22.7
MAX INSP.	20.0	17.5	28.6	10.04	4.15	41.3	—
MAX EXP.	20.0	16.7	26.2	8.75	2.05	23.4	—



TEXT-FIG. 27.

No. 27 (Case 4317).—Male, laborer; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 12 months ago with severe cough. Later fever and chills. Slight pain in chest. Tendency to tire easily. His general condition has remained poor under sanatorium treatment with the lung condition progressive.

	kg.
Height 178 cm. Theoretical normal weight	70.5
Present weight	59.0
Patient's idea of normal weight	71.5
Date of highest weight 11 months ago	71.5
" " lowest " 8 " "	57.5

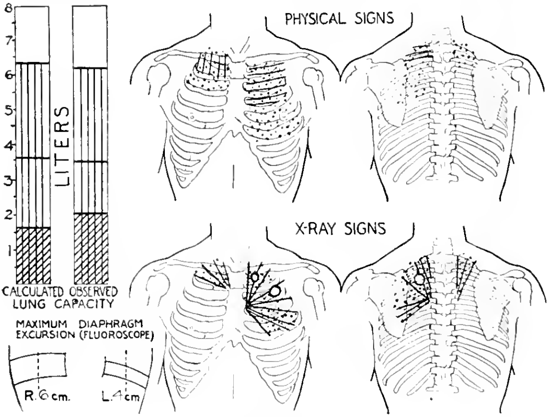
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Marked dullness at right apex to the second rib. Moderate dullness at right apex posteriorly. Harsh breath at right apex to the second rib. Increased breath sounds at left apex to the third rib. Breath sounds increased at both apices posteriorly. Medium moist râles on cough in right lung from apex to fourth rib anteriorly, and from apex to below the angle of the scapula posteriorly. Medium moist râles on cough at left apex to the second rib anteriorly; below this fine râles to the base. Posteriorly medium moist râles on cough from the apex to an inch above the angle of the scapula.

X-Ray Signs.—April 7, 1917. Right apex perfectly clear. First, second, and third interspaces densely infiltrated. The apex may be excavated, but does not give physical signs of cavity. There is a small cavity 1 cm. in diameter at the inner end of the first interspace. Left apex and first and second interspaces densely infiltrated and spotted. Third and fourth interspaces slightly infiltrated and spotted. Mediastinal contents normally placed.

No. 28 (CASE 4346)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	19.9	18.3	26.5	9.65	3.5	36.2	44.1
MAX INSP.	19.9	18.7	28.5	11.60	6.2	53.5	——
MAX EXP.	19.9	17.1	25.3	8.60	2.0	23.3	——



TEXT-FIG. 28.

No. 28 (Case 4346).—Male, pattern maker; age 31 years. Advanced; inactive. Sputum + on admission.

Onset 31 months ago with fever, chills, and night sweats. Cough and expectoration moderate. Rectal fistula. Anorexia, hoarseness, and return of fever 5 months ago. Loss of 4.5 kg. in weight since. During his stay in the hospital his general condition has been unchanged and his lung condition about the same.

	kg.
Height 174 cm. Theoretical normal weight	69.0
Present weight	61.5
Patient's idea of normal weight	62.5
Date of highest weight 14 months ago	67.0
" " lowest " 1 month "	58.0

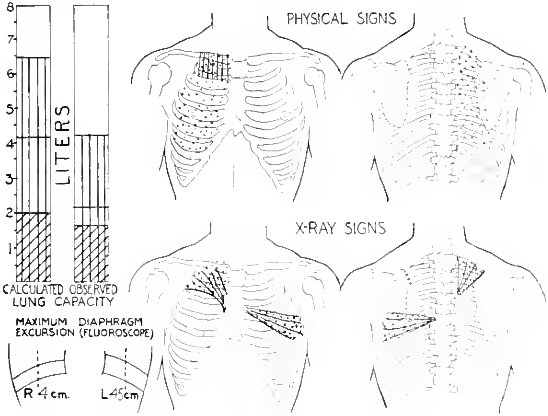
Treatment duration 1 month.

Physical Signs.—April 9, 1917. Slight dullness at right apex to the second rib. Dullness at upper part of left lung from the second to the fourth ribs. Left apex dull posteriorly. The breath sounds are increased from the clavicle to the fourth rib on the left side. Medium moist râles on cough at the right apex to the third rib. Medium moist râles on cough at the right apex posteriorly. Medium moist râles on cough from the clavicle to the sixth rib on the left side, and posteriorly from the apex to a point midway between the spine and angle of the scapula.

X-Ray Signs.—April 7, 1917. Right, moderate infiltration, spotting, and striation of the apex and first interspace. Left, moderate spotting and striation of the apex. Slight spotting of the first interspace. Dense spotting in the second interspace; cavity 3 by 5 cm. in the outer half. Third interspace moderately spotted and striated. Fourth interspace densely spotted and striated. Small cavity under clavicle. Mediastinal contents markedly to the left. Right lung area greatly increased.

No. 29 (CASE 3952)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	21.3	19.3	27.2	11.2	2.14	19.1	23.2
MAX INSP.	21.3	19.6	28.2	11.8	4.24	35.9	——
MAX EXP	21.3	18.7	26.4	10.5	1.64	15.6	——



TEXT-FIG. 29.

No. 29 (Case 3952).—Male, jeweler; age 20 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 16 months ago with cough; severe from the beginning and unaccompanied by other symptoms except slight expectoration later. His general condition under sanatorium treatment has remained good; lung disease increased.

	kg.
Height 174 cm. Theoretical normal weight	66 0
Present weight	77 0
Patient's idea of normal weight	68 0
Date of highest weight 4 months ago	78 0
" " lowest " 18 " "	68 0

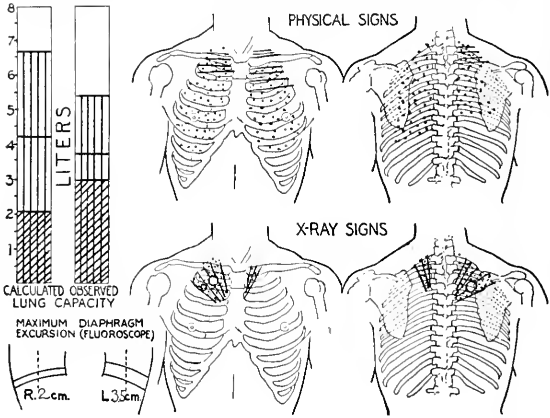
Treatment duration 12 months.

Physical Signs.—April 9, 1917. Impaired resonance on percussion over the right apex anteriorly to the second rib. Diminished breath sounds over the entire left lung, anteriorly and posteriorly. Coarse râles on cough in right lung anteriorly from apex to base. Posteriorly fine râles on cough from apex to angle of scapula. Coarse râles on cough in left lung from the clavicle above to the sixth rib below. None heard posteriorly.

X-Rays Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left, fifth and sixth interspaces moderately densely infiltrated. Mediastinal contents normal.

No. 30 (CASE 4027)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	21.4	20.2	26.0	11.55	3.7	32.1	21.2
MAX INSP.	21.4	20.6	27.4	12.10	5.4	44.6	——
MAX EXP.	21.4	19.9	25.7	11.00	3.0	27.3	——



TEXT-FIG. 30.

No. 30 (Case 4027).—Male, farmer; age 27 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 24 months ago with cough. Expectoration began 7 months later. Several small hemoptyses; moderate dyspnea. General condition bettered by sanatorium treatment; lung condition slightly progressive.

	kg.
Height 179 cm. Theoretical normal weight	74.5
Present weight	66.0
Patient's idea of normal weight	65.5
Date of highest weight 5 months ago	68.0
" " lowest " 20 " "	61.0

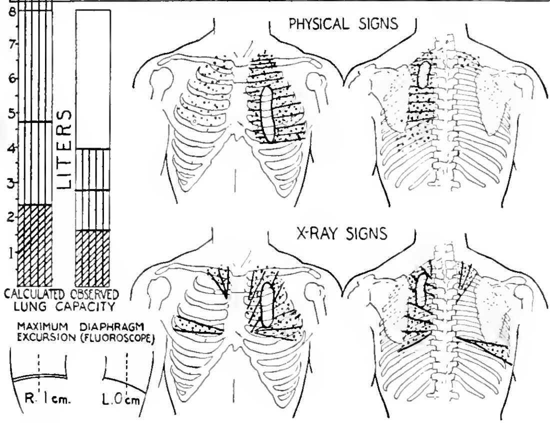
Treatment duration 11 months.

Physical Signs.—April 9, 1917. Moderate dullness over right apex anteriorly to the second rib, posteriorly to the spine. Moderate dullness over left lung anteriorly to the third rib; posteriorly no marked change. Breath sounds of upper part of right lung harsh at apex, bronchial in second interspace. Breath sounds increased in upper part of left lung to the third rib anteriorly. Coarse râles on cough in right lung to base anteriorly; posteriorly medium râles on cough from apex to angle of scapula Medium râles on cough in left lung from clavicle above to the base; posteriorly fine râles on cough from apex to an inch below the angle of the scapula.

X-Ray Signs.—April 7, 1917. Right apex and first, second, and third interspaces very densely spotted and infiltrated. Moderate sized cavity in the first interspace. Left apex is moderately spotted. Mediastinal contents are normal.

No. 31 (CASE 4130)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. / CHEST VOL.	RATIO 100 X VITAL CAP. / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	22.0	19.7	29.3	12.70	2.82	22.2	18.9
MAX INSP.	22.0	23.0	30.6	15.50	4.02	25.9	——
MAX EXP.	22.0	19.3	28.6	12.15	1.62	13.3	——



TEXT-FIG. 31.

No. 31 (Case 4130).—Male, sheet metal worker; age 25 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 16 months ago with cold. Cough moderate; expectoration profuse; slight loss in weight; later chills and afternoon temperature of 100° F. Has been continuously toxic during stay in sanatorium; lung lesion progressive.

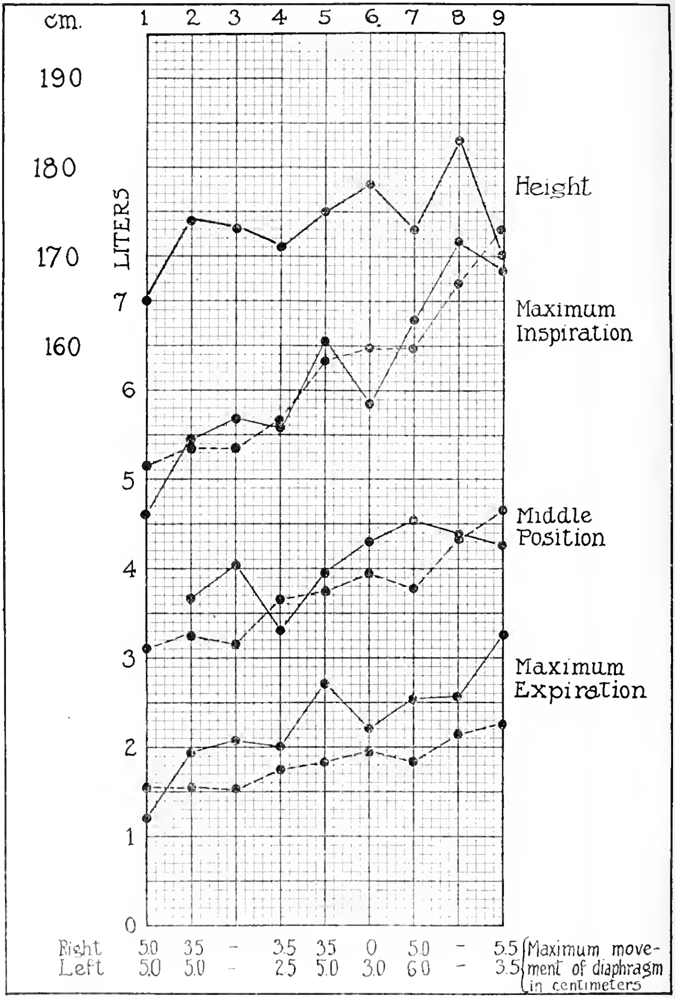
	kg.
Height 177 cm. Theoretical normal weight	68.5
Present weight	?
Patient's idea of normal weight	72.5
Date of highest weight 4 to 5 years ago	76.0
" " lowest " 12 months "	66.5

Treatment duration 8 months.

Physical Signs.—April 9. 1917. Dullness anteriorly over the left lung from apex to the sixth rib. Cracked pot percussion in the second, third, fourth, and fifth interspaces. Dullness posteriorly over the left lung from the apex to the angle of the scapula. Breath sounds harsh posteriorly at right apex. No great change in right lung anteriorly. Diminished breath sounds in left lung posterior to base. Anteriorly slightly increased at left apex, cavernous in the third, fourth, and fifth interspaces.

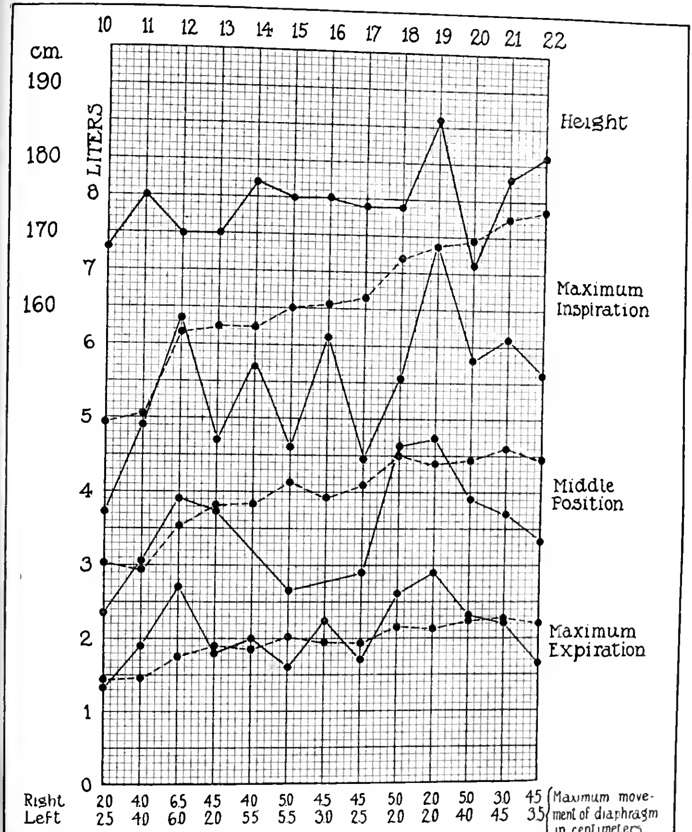
Râles: Right apex to second rib on cough medium moist râles, second to fourth sibilant. Posteriorly medium moist on cough from apex to the spine of the scapula. Fine and medium moist râles on cough in left lung anteriorly and posteriorly from apex to base.

X-Ray Signs.—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Fourth interspace moderately densely infiltrated. Entire left lung densely spotted. Large cavity, 14 by 5 cm. Mediastinal contents markedly to the left. Right lung area greatly increased.

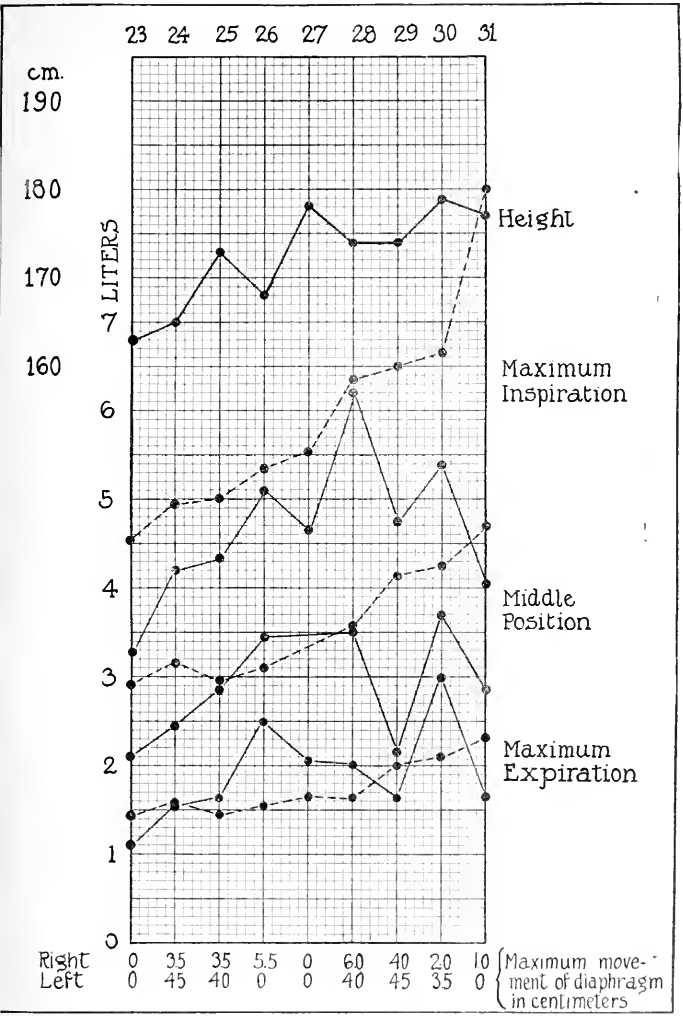


In the charts the numbers below indicate the maximum excursion of the right and left diaphragm. The numbers above the chart refer to the individual diagrams and descriptions (Text-figs. 1 to 31).

TEXT FIG. 32. Lung volumes in men with incipient pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



TEXT-FIG. 33. Lung volumes in men with moderately advanced pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



TEXT-FIG. 34. Lung volumes in men with advanced pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.

STUDIES OF LUNG VOLUME.

III. Tuberculous Women.

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INTRODUCTION.

In the preceding paper (Garvin, Lundsgaard, and Van Slyke) a report was made of a series of determinations of the different lung volumes in thirty-one adult men suffering from pulmonary tuberculosis. A comparison was drawn between the actual values and the values calculated from the chest dimensions on the basis of certain ratios previously worked out on normal subjects (Lundsgaard and Van Slyke). The literature concerning pulmometry in pulmonary tuberculosis is given in the preceding paper.

The present paper is a report of similar determinations on twenty adult women with phthisis. The technique in determining the lung volumes, in measuring the chest wall, and in determining the movement of the diaphragm is fully described in Papers I and II. The presentation of the experimental and clinical observations on the women is carried out after exactly the same plan as in Paper II, where sufficient explanation can be found. Only the explanation of the symbols used in the individual diagrams (Text-figs. 1 to 20) will be repeated here:

Physical Signs.—
Light lines, slight dullness.
Heavy lines, moderate dullness.
Cross-hatching, marked dullness.
Fine dots, fine râles.
Larger dots, moderate and coarse râles.

Small rings, large crackling râles.
Crosses, pleuritic rubs.
Circles, antrum formation.

There is no difference in the interpretation of horizontal and vertical lines

X-Ray Signs.—

Lightly shaded lines, slight density of shadow.
Heavy lines, marked density.
Circles, cavity.
Dots, stippling, the larger the dots, the coarser the stippling.

There is no difference in the interpretation of horizontal and vertical lines.

The patients are divided into three groups, the incipient (Text-fig. 21), moderately advanced, and advanced cases. The last two groups, however, are described together and the values put together in Text-fig. 22. Nos. 9 and 19 are advanced (Group III in Paper II); the rest are moderately advanced (Group II in Paper II). The reason for this is that there is, as pointed out in Paper I, no sharp difference in our results between patients belonging to Groups II and III.

Group I. Incipient Cases (Nos. 1 to 8, Text-Fig. 21).

It will be remembered that the result of the determinations on nine men with incipient tuberculosis was (1) a normal total capacity, (2) a moderately increased residual air resulting in (3) a moderately diminished vital capacity. At first sight the results on the women seem quite opposite. The values in Nos. 1, 2, 3, 4, and 8 agree with those found in the men, but in Nos. 5, 6, and 7, although they also are clinically incipient, a great decrease in the total and vital capacity is encountered. A similar drop is seen in the middle capacity, whereas the residual air is normal.

However, sufficient cause can be found to account for this. No. 5 was a patient with miliary tuberculosis of the lungs. She had râles on both sides all over the lungs. No. 6 had a bronchial stenosis on the left side; the left lung participated only to a small extent in the ventilation. It will be seen that her left diaphragm moved in the opposite direction to the normal movement in respiration. This probably was a result of the difficult passage to the left lung. The observed vital and middle capacity is about half of the calculated. No. 7 did not move her diaphragm at all. The residual air, however,

is not increased, which probably can be looked upon as indicating that her diaphragm is fixed in expiratory position. The inability to lower the diaphragm at inspiration must, of course, diminish her total capacity to a considerable extent.

The results of the determinations in these cases therefore confirm the previous findings in men, and show that if the total capacity is diminished in patients with incipient tuberculosis, some special cause is to be found, such as miliary tuberculosis, obstruction of bronchi, or inability to move the diaphragm.

Group II. Moderately Advanced and Advanced Cases (Nos. 9 to 20, Text-Fig. 22).

Group II (Nos. 9 to 20, moderately advanced and advanced cases) shows the same picture that was found in men: (1) As a rule diminished total capacity. In all the cases except Nos. 9, 10, 13, and 17, the total capacity is below the normal minimum; in these four cases it is above the normal minimum but below the normal average. (2) Decreased vital capacity. (3) Fairly normal residual air. (4) The middle capacity (not determined in all cases) is in some patients normal, in others subnormal. As mentioned before, we do not lay much stress on the determination of the middle capacity, because it is dependent not only on anatomic but also on functional factors. What the latter are we do not understand, but we have seen subjects unconsciously inflate or deflate the chest so as to change the middle capacity by several hundred centimeters.

Excursions of the Diaphragm.

The technique is described in Papers I and II. The excursions of the diaphragm are, as a whole, smaller than in normal subjects and agree with those found in the men. In one instance (No. 7) no movement was found at all. No evidence was found for a mechanical obstacle in the pleura, lungs, or abdomen. Whether the diaphragm was paralyzed through involvement of the phrenic nerves, or whether it was due to a reflex, we do not know. In another case (No. 6) the left half of the diaphragm moved in the direction opposite to the normal. Sufficient explanation is found in the fact that the left

TABLE I.

Influence of Change of Position and of Exercise on Pulse and Respiration.

No. on individual diagrams	Case No.	Resting in bed.		Standing up.		After having run up three flights of stairs.		
		Pulse.	Respirations.	Pulse.	Respirations.	Pulse.	Respirations.	Other symptoms.
Group I.								
1	4167	94	24	120	24	124	30	Very slight dyspnea. Pulse slows quickly.
2	4247	90	24	96	24	150	26	Slight dyspnea; cyanosis of hands.
3	4164	96	18	110	25	136	32	Palpitation; dyspnea; throbbing temples.
4	4215	74	18	90	24	150	44	Very nervous; very dyspneic; face flushed; hands cyanotic.
5	4190	102	30	112	34	150	42	Palpitations; dizziness.
6	4151	74	24	80	24	130	32	Very dyspneic; face slightly flushed.
7	4309	102	30	116	30	160	36	Slight dyspnea; face flushed.
8	3996	78	18	102	18	120	24	Face flushed.
Group II.*								
9	4061	80	17	115	18	145	22	None.
10	4314	72	18	96	20	132	28	Slight dyspnea; face slightly flushed.
11	4059	102	22	120	22	140	30	Slight dyspnea; face much flushed.
12	3882	72	26	84	24	150	30	Face flushed; dizziness; very dyspneic.
13	4191	72	16	102	16	160	20	None.
14	4044	90	24	110	22	147	26	Flush.
15	4192	72	22	84	22	150	30	Moderate dyspnea; face slightly pale.
17	4283	90	18	92	18	125	28	Palpitation; face flushed.
18	4264	90	12	100	16	120	24	"
19	3908	112	22	112	22	145	28	" marked dyspnea; face flushed; weak pulse.
20	4103	64	16	75	20	145	28	Slight dyspnea; face pale; pulse very weak.

* Group II is composed of moderately advanced cases (Group II in Paper II) and advanced cases (Group III in Paper II).

main bronchus was almost obstructed. An inspiratory movement of the thoracic wall could, under these conditions, result in an upward movement of the diaphragm on this side.

Influence of Change of Position and of Exercise on Pulse and Respiration (Table I).

The increase in pulse rate on exercise is still more marked in the women than in the men. The rate of respiration is also somewhat increased. As a whole, in men no such increase in the respiration was found.

SUMMARY.

The total capacity, middle capacity, and residual air have been determined in twenty adult women suffering from pulmonary tuberculosis. The chest volumes have been determined in each case and the normal lung volumes calculated by means of the ratios worked out in Paper I and applied to thirty-one men in Paper II. The excursions of the diaphragm have been determined by fluoroscopy in all cases.

Of eight patients with incipient tuberculosis, five had lung capacities like those of men in the same group;[1] *i.e.*, about normal total capacity, slightly increased residual air, and consequently somewhat decreased vital capacity. Three had considerably diminished total capacity. In these three patients, however, clinical abnormalities were found (extensive miliary tuberculosis, obstruction of bronchus, fixation of diaphragm in expiratory position).

In twelve patients with moderately advanced and advanced tuberculosis, the results agreed with those found in men,[1] the total capacity and vital capacity being decreased, while the residual air was practically normal.

BIBLIOGRAPHY.[2]

Garvin, A., Lundsgaard, C., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxvii, 87.

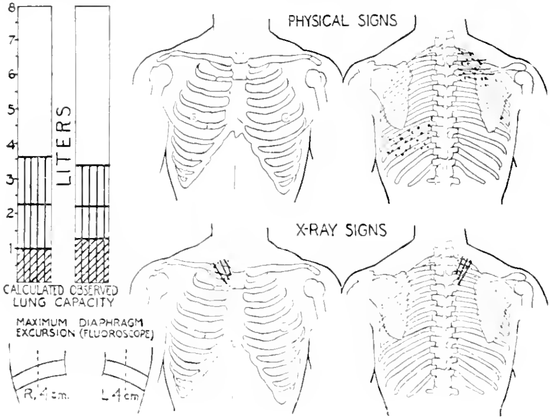
Lundsgaard, C., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxvii, 65.

[1] See Paper II.

[2] An extensive bibliography can be found in the two preceding papers.

No. 1 (CASE 4167)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm	cm.	cm	liters	liters		
REST	16.8	16.1	22.2	6.00	2.15	35.8	35.0
MAX INSP.	16.8	17.3	22.6	6.56	3.35	51.0	——
MAX EXP.	16.8	15.1	21.5	5.45	1.25	23.0	——



TEXT-FIG. 1.

No. 1 (Case 4167).—Female, factory worker; age 22 years. Incipient; active. Sputum — — +, on admission, in course of treatment, and at present.

Onset 17 months ago with slight cough, blood-streaked sputum, and loss of weight and strength. Under treatment has gained weight but disease is still slightly active. Physical signs have increased slightly in the past 7 months, and a previous negative sputum has become positive.

		kg.
Height 157 cm.	Theoretical normal weight	53.5
Present weight		48.0
Patient's idea of normal weight		43.0
Date of highest weight	2 months ago	48.5
" " lowest	" 12 " "	41.0

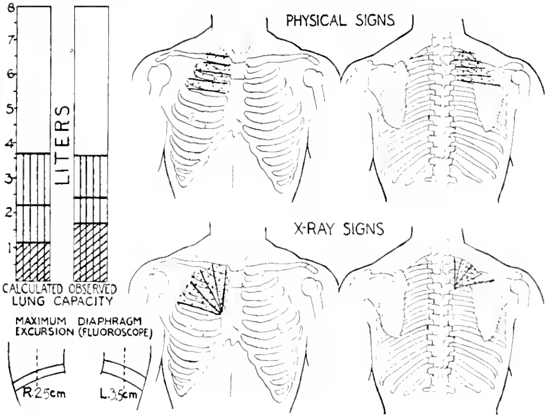
Treatment duration 7 months.

Physical Signs.—April 9, 1917. Right, no impairment of resonance; breathing harsh; fine râles with cough only posteriorly to fourth spine. Left, upper lobe normal; posteriorly there are moist râles from base to ninth dorsal spine with aid of cough; no dullness or breath sound change in this area, and signs are intrapulmonic (not affected by breathing and diaphragm has a normal excursion). (See illustration of normal volume.)

X-Ray Signs.—April 7, 1917. Right apex moderately densely infiltrated. Root slightly infiltrated. Left lung practically normal (see physical examination). Mediastinal contents normal.

No. 2 (CASE 4247)

POSITION	CHEST DIMENSIONS: STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"	LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	cm	cm.	cm	liters	liters		
REST	15.0	16.8	23.8	6.00	2.4	40.0	25.0
MAX INSP.	15.0	17.4	25.5	6.65	3.6	54.2	——
MAX. EXP.	15.0	16.0	23.7	5.69	1.7	29.9	——



TEXT-FIG. 2.

No. 2 (Case 4247).—Female, student; age 18 years. Incipient; inactive. Sputum − ± +, on admission, in course of treatment, and at present.

Onset 16 months ago with cough and blood-streaked sputum. Under sanatorium treatment symptoms have disappeared and physical signs have diminished in number.

	kg.
Height 156 cm. Theoretical normal weight	52.0
Present weight	64.0
Patient's idea of normal weight	59.0
Date of highest weight 0 months ago	64.0
" " lowest " 4 " "	58.0

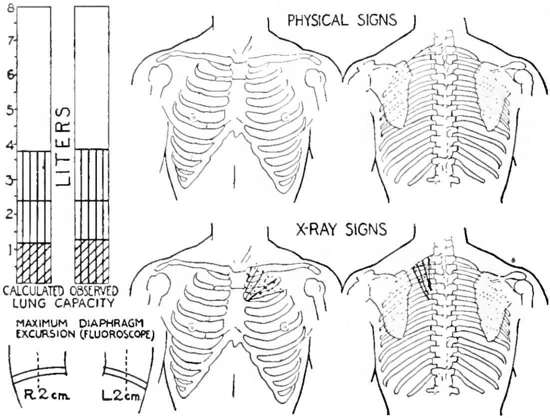
Treatment duration 5 months.

Physical Signs.—April 9, 1917. Right, slight impairment of resonance; breath sounds are a little harsh; fine râles appear with aid of cough to the third rib anteriorly and fourth spine posteriorly. Left lung seems normal.

X-Ray Signs.—April 7, 1917. Right apex and first, second, and third interspaces very slightly stippled and striated. Left lung normal. Mediastinal contents are a little to the right. Left lung area increased.

No. 3 (CASE 4164)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	15.8	15.3	26.4	6.38	2.40	36.6	41.5
MAX INSP.	15.8	16.2	27.4	6.96	3.90	56.0	——
MAX EXP.	15.8	14.9	25.7	6.06	1.25	20.6	——



Text-Fig. 3.

No. 3 (Case 4164).—Female, domestic; age 19 years. Incipient; inactive. Sputum + ± −, on admission, in course of treatment, and at present.

Onset 12 months ago with malaise and loss of strength; slight cough with streaked sputum for a few days. Under sanatorium treatment symptoms have diminished and physical signs have disappeared.

	kg.
Height 152 cm. Theoretical normal weight	51.0
Present weight	55.0
Patient's idea of normal weight	50.0
Date of highest weight 1 month ago	56.5
" " lowest " 7 months "	47.5

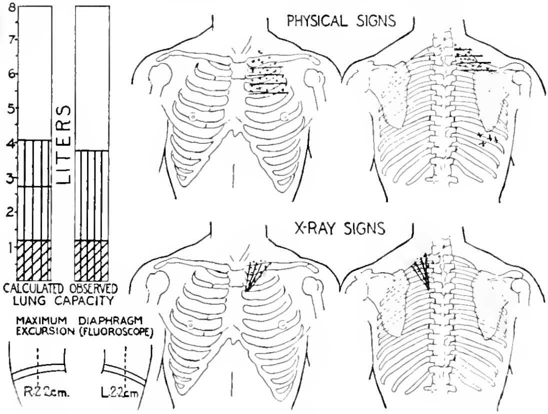
Treatment duration 7 months.

Physical Signs.—April 9, 1917. Right lung seems normal. Left lung seems normal. Patient has had tubercle bacilli in sputum within 6 weeks.

X-Ray Signs.—April 7, 1917. Right lung clear. Left apex stippled and striated moderately to the second rib. Mediastinal contents normal.

No. 4 (CASE 4215)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	16.7	17.2	23.4	6.71	2.52	37.0	36.0
MAX INSP.	16.7	18.4	24.5	7.54	3.72	49.1	——
MAX EXP.	16.7	16.3	22.5	6.12	1.22	19.9	——



TEXT-FIG. 4.

No. 4 (Case 4215).—Female, student; age 18 years. Incipient; inactive. Sputum + + −, on admission, in course of treatment, and at present.

Onset 30 months ago with cough following influenza. Under sanatorium treatment symptoms have disappeared, and physical signs diminished about 75 per cent.

	kg.
Height 155 cm. Theoretical normal weight	51.0
Present weight	61.0
Patient's idea of normal weight	50.0
Date of highest weight 0 months ago	61.0
" " lowest " 30 " "	47.0

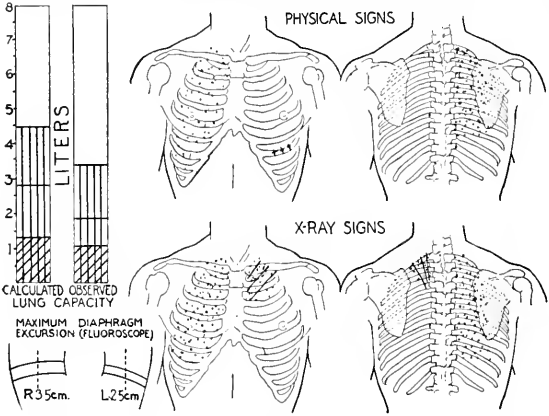
Treatment duration 7 months.

Physical Signs.—April 9, 1917. Left apex, slight dullness; breath sounds diminished; Râles fine and moist to third rib anteriorly and to spine of scapula posteriorly. Right base posteriorly gives fine friction rubs on deep breathing.

X-Ray Signs.—April 7, 1917. Right lung fairly normal. Left apex and first interspace moderately densely infiltrated. Mediastinal contents normal.

No. 5 (CASE 4190)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	17.7	16.7	25.7	7.60	1.82	24.0	30.5
MAX INSP.	17.7	17.4	26.3	8.10	3.32	41.0	—
MAX EXP.	17.7	15.7	24.9	6.92	1.02	14.7	—



TEXT-FIG. 5.

No. 5 (Case 4190).—Female, stock clerk; age 17 years. Incipient; inactive. Chronic miliary tuberculosis of the right lung. Sputum — —, on admission and in course of treatment.

Onset 7 months ago with slight cough and malaise, and dyspnea on exertion. Under treatment has improved in symptoms while the signs have remained the same.

		kg.
Height 158 cm.	Theoretical normal weight	53.5
Present weight		59.0
Patient's idea of normal weight		52.0
Date of highest weight	0 months ago	59.0
" " lowest	" 17 " "	45.5

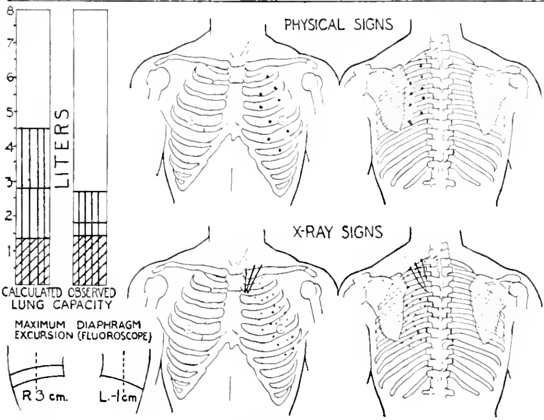
Treatment duration 6 months.

Physical Signs.—April 9, 1917. Right, no impairment of resonance or change in breath sounds; a few râles after cough fairly well disseminated throughout the right lung. Left, no change in resonance or in breath sounds; a few friction rubs at the base.

X-Ray Signs.—April 7, 1917. Right lung shows very fine discrete spotting throughout. Left apex and first and second interspaces show slight spotting and striations. Mediastinal contents normal.

No. 6 (CASE 4151)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm	cm.	cm	liters	liters		
REST	18.2	17.6	23.5	7.52	1.86	24.7	18.0
MAX INSP.	18.2	18.0	24.7	8.15	2.74	33.6	——
MAX EXP.	18.2	16.9	22.9	7.04	1.41	20.2	——



TEXT-FIG. 6.

No. 6 (*Case 4151*).—Female, student; age 19 years. Incipient; inactive. Left primary bronchus obstruction (large gland). Sputum + + −, on admission, in course of treatment, and at present.

Onset 20 months ago with malaise, slight cough, and dyspnea. Under treatment she has gained much in weight, is still dyspneic, but feels much better. Physical signs unchanged.

	kg.
Height 158 cm. Theoretical normal weight	52.5
Present weight	52.5
Patient's idea of normal weight	54.5
Date of highest weight 22 months ago	56.5
" " lowest " 7 " "	48.5

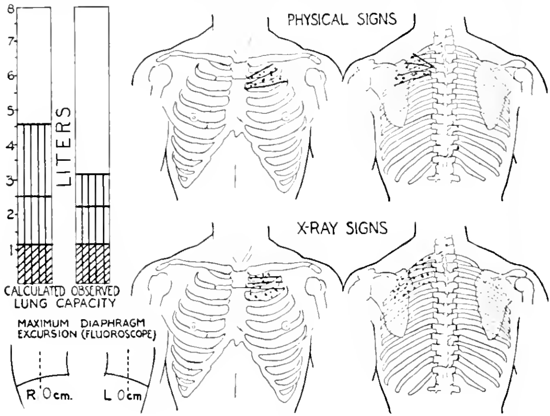
Treatment duration 7 months.

Physical Signs.—April 9, 1917. Right, chest normal; no impairment of resonance. Left, breathing diminished; coarse loud râles throughout left chest.

X-Ray Signs.—April 7, 1917. Right lung a little hazy throughout. Left lung, apical space slightly infiltrated; rest of the lung shows very fine slight spotting on full inspiration. Mediastinal contents are to left. Right lung area much increased.

No.7 (CASE 4309)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	16.9	16.7	23.7	6.60	2.20	33.0	29.5
MAX INSP	16.9	18.7	26.2	8.30	3.10	37.3	——
MAX EXP	16.9	15.5	23.1	6.04	1.15	19.0	——



TEXT-FIG. 7.

No. 7 (Case 4309).—Female, student; age 16 years. Incipient; inactive. Sputum + + −, on admission, in course of treatment, and at present.

Onset 8 months ago with malaise and slight cough. Under treatment symptoms and signs have markedly diminished. Patient feels perfectly well.

	kg.
Height 157 cm. Theoretical normal weight	52.0
Present weight	48.5
Patient's idea of normal weight	45.5
Date of highest weight 0 months ago	48.5
" " lowest " 15 " "	45.5

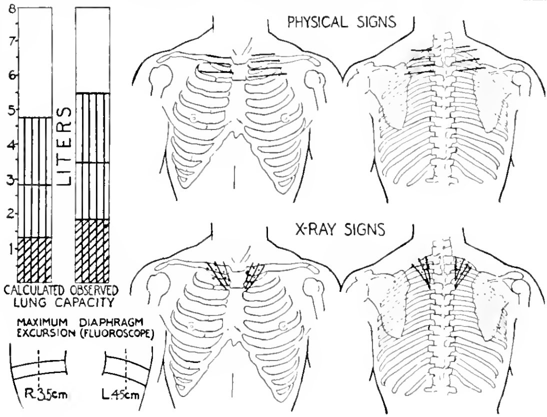
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Right lung normal. Left, slight dullness below clavicle; breath sounds are a little harsh; râles over second and third interspaces anteriorly and to fourth spine posteriorly with aid of cough.

X-Ray Signs.—April 7, 1917. Right lung normal. Left, first and second interspaces moderately densely infiltrated. Mediastinal contents normal.

No. 8 (CASE 3996)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	18.7	16.7	24.4	7.61	3.42	45.0	46.7
MAX INSP.	18.7	18.5	25.1	8.68	5.42	62.2	——
MAX EXP.	18.7	16.0	23.4	7.00	1.87	26.7	——



TEXT-FIG. 8.

No. 8 (Case 3996).—Female, trained nurse; age 35 years. Incipient; inactive. Sputum + ± −, on admission, in course of treatment, and at present.

Onset 25 months ago with malaise and cough. Under sanatorium treatment general condition much improved.

	kg.
Height 172 cm. Theoretical normal weight	68.0
Present weight	67.5
Patient's idea of normal weight	66.0
Date of highest weight 0 months ago	67.5
" " lowest " 6 " "	59.0

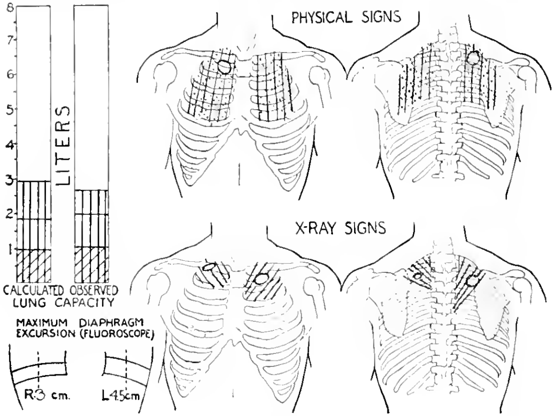
Treatment duration 11 months.

Physical Signs.—April 9, 1917. Right, slight impairment of resonance; slight increase in sharpness of breath sounds; no râles. Left, slight dullness; breath sounds slightly increased in intensity; no râles.

X-Ray Signs.—April 7, 1917. Right apex moderately densely infiltrated and spotted; rest of lung normal. Left apex moderately densely infiltrated and spotted; rest of lung normal. Mediastinal contents slightly to the left.

No. 9 (CASE 4061)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 × LUNG CAP / CHEST VOL	RATIO 100 × VITAL CAP / CHEST VOL
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm.	liters	liters		
REST	15.5	15.8	20.8	5.09	1.95	38.2	32.4
MAX INSP.	15.5	17.0	21.3	5.34	2.60	48.7	——
MAX EXP.	15.5	15.2	20.3	4.78	1.0	20.9	——



TEXT-FIG. 9.

No. 9 (Case 4061).—Female, saleswoman; age 34 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 48 months ago with malaise, cough, expectoration, and slight loss of weight. Under sanatorium treatment general condition has slightly improved, the physical signs remaining the same.

	kg.
Height 160 cm. Theoretical normal weight	58.5
Present weight	45.5
Patient's idea of normal weight	50.0
Date of highest weight 6 years ago	50.0
" " lowest " 12 months ago	40.5

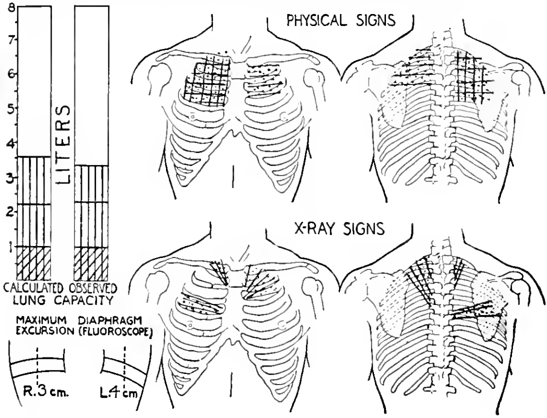
Treatment duration 10 months.

Physical Signs.—April 9, 1917. Right, slight dullness; breath sounds are moderately harsh; râles occur at apex to fifth rib anteriorly and to sixth spine posteriorly with aid of cough; cavernous breathing at right apex. Left, moderate dullness; breath sounds are markedly harsh; râles of medium moist type to fifth rib anteriorly and to sixth spine posteriorly, greatly increased by aid of cough.

X-Ray Signs.—April 7, 1917. Right apex and first interspace densely infiltrated; cavity under the clavicle $1\frac{1}{2}$ by $3\frac{1}{2}$ cm. Left apex and first and second interspaces quite densely infiltrated; cavity below the clavicle 4 by 3 cm. Mediastinal contents centrally placed.

No. 10 (CASE 4314)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm	cm.	liters	liters		
REST	18.0	14.6	22.1	5.81	2.25	38.8	40.5
MAX INSP.	18.0	14.9	24.0	6.43	3.35	52.1	——
MAX EXP.	18.0	13.9	21.5	5.38	1.0	18.6	——



TEXT-FIG. 10.

No. 10 (Case 4314).—Female, housewife; age 30 years. Moderately advanced; active. Sputum — — —, on admission, in course of treatment, and at present.

Onset 16 months ago with malaise, cough, and expectoration; loss of strength followed; slight temperature. Under treatment symptoms became stationary, but lesion has progressed slightly.

	kg.
Height 155 cm. Theoretical normal weight	53.5
Present weight	40.5
Patient's idea of normal weight	47.5
Date of highest weight 8 years ago	47.5
" " lowest " 3 months ago	38.5

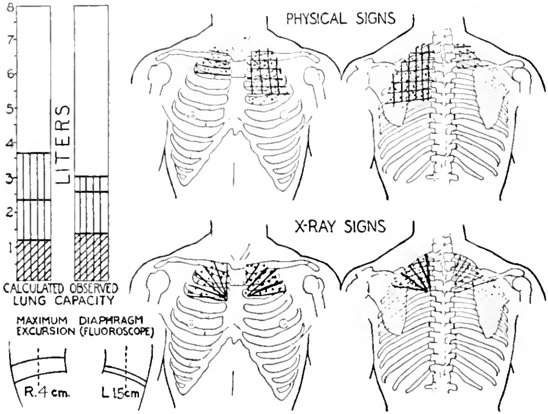
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Right, marked impairment of resonance; breath sounds harsh; coarse moist râles on breathing, with an increase in number with cough to fourth rib anteriorly and sixth spine posteriorly. Left, slight impairment of resonance; no change in breath sounds; fine moist râles increased by cough to third rib anteriorly and fourth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex densely spotted; first and second interspaces clear; third interspace moderately densely spotted. Left apex and first and second interspaces moderately densely spotted. Mediastinal contents normal.

No. 11 (CASE 4059)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	18.3	15.9	21.7	6.32	2.5	39.6	27.0
MAX INSP.	18.3	16.4	22.3	6.68	3.0	44.9	——
MAX EXP.	18.3	14.9	21.0	5.73	1.3	23.6	——



TEXT-FIG. 11.

No. 11 (Case 4059).—Female, stenographer; age 20 years. Moderately advanced; active. Sputum − + +, on admission, in course of treatment, and at present.

Onset 18 months ago with cough, malaise, and loss of weight. Under sanatorium treatment general condition improved and symptoms diminished markedly although physical signs increased.

	kg.
Height 160 cm. Theoretical normal weight	54.5
Present weight	46.0
Patient's idea of normal weight	45.5
Date of highest weight 3 months ago	48.5
" " lowest " 10 " "	37.5

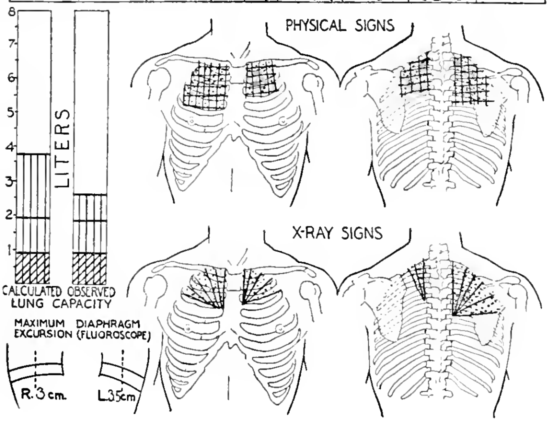
Treatment duration 10 months.

Physical Signs.—April 9, 1917. Right, slight dullness; breath sounds slightly harsh; fine moist râles to second rib anteriorly and to third spine posteriorly, much increased with cough. Upper part of left lung, marked dullness at apex to third rib anteriorly, and to sixth spine posteriorly; breath sounds amphoric anteriorly, harsh posteriorly; medium moist râles to fourth rib anteriorly and to fifth spine posteriorly. Cough increased the number of signs.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately spotted. Left apex and first interspace very dense; second interspace very finely stippled. Mediastinal contents entirely to the left. Right lung area much increased.

No. 12 (CASE 3882)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	14.1	15.4	23.6	5.12	1.8	35.3	31.5
MAX INSP.	14.1	16.4	25.1	6.80	2.6	38.2	——
MAX EXP.	14.1	14.8	23.2	4.84	0.9	18.6	——



TEXT-FIG. 12.

No. 12 (Case 3882).—Female, clerk; age 21 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset with cough 3 years ago; malaise and loss of weight followed. Later a high temperature with right base fluid which cleared up spontaneously in 60 days. Under treatment, symptoms have markedly improved and lesion has become stationary.

	kg.
Height 157 cm. Theoretical normal weight	53.5
Present weight	55.0
Patient's idea of normal weight	53.5
Date of highest weight 1913	56.0
" " lowest " 16 months ago	46.0

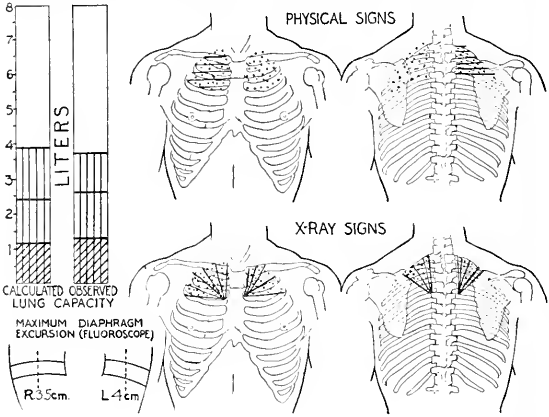
Treatment duration 15 months.

Physical Signs.—April 9, 1917. Right, marked dullness to fourth rib; harsh breathing; medium moist râles numerous with, and fewer without aid of cough to fourth rib anteriorly and sixth spine posteriorly. Left, marked dullness to third rib; harsh breathing; râles are rather moist, increased by cough, to third rib anteriorly and fourth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely stippled and striated. Left apex and first interspace moderately densely stippled and striated. Mediastinal contents normal.

No. 13 (CASE 4191)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT.-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	16.5	16.4	23.9	6.46	2.6	40.3	38.6
MAX INSP.	16.5	17.6	24.8	7.20	3.7	51.4	——
MAX EXP.	16.5	15.0	23.2	5.74	1.2	20.9	——



TEXT-FIG. 13.

No. 13 (Case 4191).—Female, factory worker; age 18 years. Moderately advanced; active. Sputum — — +, on admission, in course of treatment, and at present.

Onset 6 months ago with hemoptysis. With sanatorium treatment symptoms markedly decreased, but physical examination shows an increase in the size of the lesion.

	kg.
Height 157 cm. Theoretical normal weight	51.0
Present weight	57.0
Patient's idea of normal weight	52.0
Date of highest weight 2 months ago	57.5
" " lowest " 11 " "	48.0

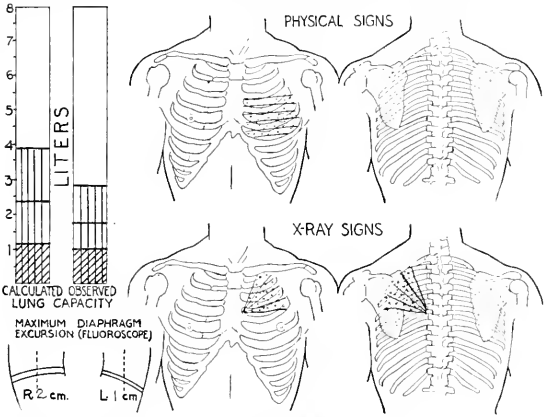
Treatment duration 6 months.

Physical Signs.—April 9, 1917. Right, slightly dull; breath sounds slightly harsh; medium moist râles to third rib anteriorly and to fourth spine posteriorly, very much increased by cough. Left, no dullness; breath sounds slightly harsh; medium moist râles to third rib anteriorly and to fourth spine posteriorly, much increased with the aid of cough.

X-Ray Signs.—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left apex and first and second interspaces moderately densely infiltrated. Mediastinal contents a little to the left.

No.14 (CASE 4044)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	17.2	16.4	23.5	6.62	1.70	25.6	27.2
MAX INSP.	17.2	17.6	24.3	7.34	2.80	38.1	——
MAX EXP.	17.2	15.8	23.0	6.25	1.0	16.0	——



TEXT-FIG. 14.

No. 14 (Case 4044).—Female, stenographer; age 17 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 17 months ago with cough and expectoration; no physical discomfort. Under treatment cough has diminished and weight increased. Physical signs have diminished markedly.

	kg.
Height 156 cm. Theoretical normal weight	50.5
Present weight	59.0
Patient's idea of normal weight	48.5
Date of highest weight 1 month ago	60.0
" " lowest " 19 months "	47.5

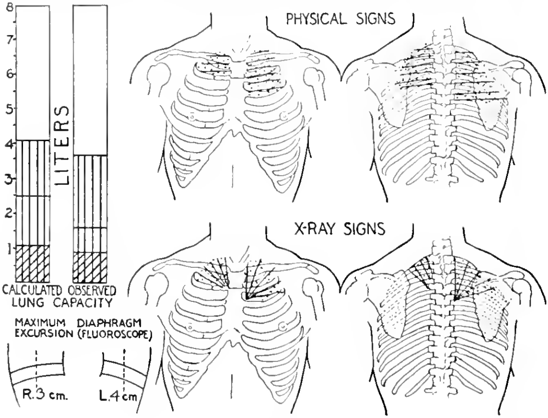
Treatment duration 11 months.

Physical Signs.—April 9, 1917. Right lung normal. Left, slight dullness over third, fourth, and fifth interspaces anteriorly; breathing harsh over this area; fine râles after cough in this area. Posteriorly there are no physical signs.

X-Ray Signs.—April 7, 1917. Right lung clear. Left, first, second, and third interspaces moderately densely infiltrated. Mediastinal contents markedly to the left. Right lung area is much increased.

No. 15 (CASE 4192)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	17.4	15.3	25.4	6.77	1.51	22.3	39.3
MAX INSP.	17.4	16.4	26.1	7.44	3.60	48.4	——
MAX EXP.	17.4	14.6	23.4	5.96	0.86	14.4	——



TEXT-FIG. 15.

No. 15 (Case 4192).—Female, clerk; age 16 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 18 months ago with cough and slight hemoptysis. Under treatment general condition improved slightly, but râle area indicates increase of disease.

	kg.
Height 163 cm. Theoretical normal weight	53.0
Present weight	55.0
Patient's idea of normal weight	50.5
Date of highest weight 2 months ago	55.5
" " lowest " 5 " "	50.5

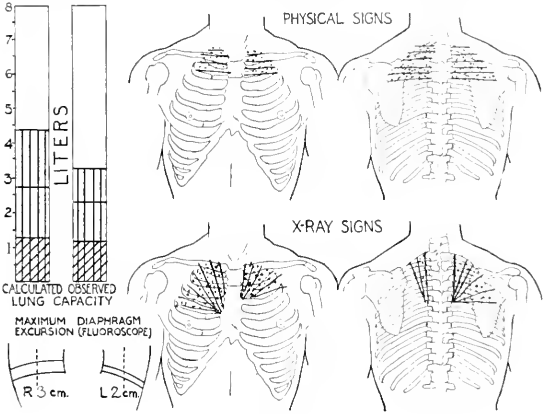
Treatment duration 6 months.

Physical Signs.—April 9, 1917. Right, slight dullness; breath sounds slightly harsh; medium fine moist râles to second rib anteriorly and sixth spine posteriorly with aid of cough. Left, slight dullness; breath sounds normal; medium moist râles at apex to third rib anteriorly and fourth spine posteriorly with aid of cough.

X-Ray Signs.—April 7, 1917. Right apex and first interspace slightly stippled and striated. Left apex and first and second interspaces moderately stippled and striated. Mediastinal contents normal.

No. 16 (CASE 3947)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.7	15.6	23.3	7.15	2.20	30.8	29.3
MAX INSP.	19.7	16.4	24.8	8.00	3.20	40.0	——
MAX EXP.	19.7	14.7	22.7	6.58	1.10	16.7	——



TEXT-FIG. 16.

No. 16 (*Case 3947*).—Female, cashier; age 20 years. Moderately advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 14 months ago with malaise, loss of weight, pleurisy, and cough with expectoration. Under sanatorium treatment general condition and weight improved, and physical signs have diminished.

	kg.
Height 156 cm. Theoretical normal weight........................	52.0
Present weight..	50.0
Patient's idea of normal weight................................	52.0
Date of highest weight 36 months ago...........................	60.0
" " lowest " 14 " "	47.0

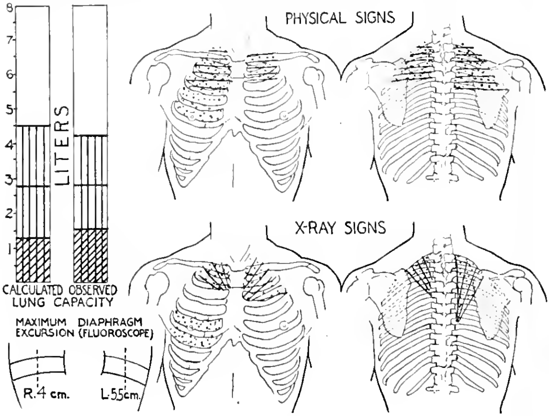
Treatment duration 12 months.

Physical Signs.—April 9, 1917. Right, slight dullness to second rib; no breath sound change; râles after cough to second rib anteriorly and fourth spine posteriorly. Left lesion gives the same signs to almost exactly the same extent. In 12 months the physical signs have diminished about one-half.

X-Ray Signs.—April 7, 1917. Right apex and first interspace moderately densely spotted and striated; second and third interspaces finely stippled. Left apex and first and second interspaces present a mixture of coarse and fine stipplings. Mediastinal contents normal.

No. 17 (CASE 4283)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	18.9	16.3	24.6	7.57	2.8	37.0	34.4
MAX INSP.	18.9	17.1	25.3	8.18	4.2	51.3	——
MAX EXP.	18.9	15.6	23.3	6.86	1.6	23.4	——



TEXT-FIG. 17.

No. 17 (Case 4283).—Female, domestic; age 39 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset insidious 6 months ago, with malaise, slight cough, and slight hemoptysis. Under sanatorium treatment symptoms disappeared and physical signs have diminished in number.

	kg.
Height 171 cm. Theoretical normal weight	67.5
Present weight	56.5
Patient's idea of normal weight	54.5
Date of highest weight 40 months ago	58.0
" " lowest " 9 " "	51.0

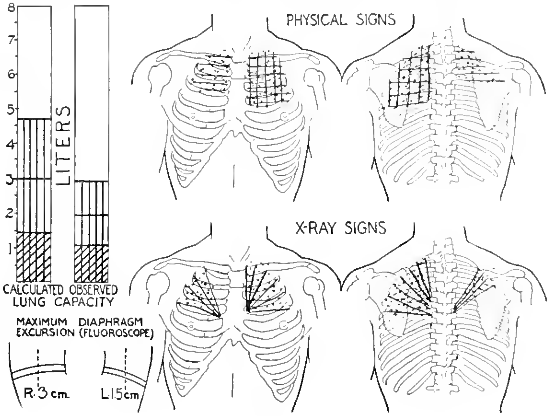
Treatment duration 3 months.

Physical Signs.—April 9, 1917. Right, resonance impaired to third rib; harsh breathing; fine râles after cough to fourth interspace anteriorly and fifth spine posteriorly. Left, resonance impaired to second rib; breathing is diminished in intensity; fine râles after cough to second rib anteriorly and fourth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex and first interspace slightly stippled and striated; the fourth and fifth interspaces are very slightly stippled. Left apex and first and second interspaces are slightly striated and stippled. Mediastinal contents centrally placed.

No. 18 (CASE 4264)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 × LUNG CAP / CHEST VOL	RATIO 100 × VITAL CAP / CHEST VOL
	STERNUM	ANT.POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.4	16.4	25.6	8.15	—	—	23.4
MAX INSP.	19.4	17.3	26.0	8.72	2.9	33.2	—
MAX EXP.	19.4	15.7	25.0	7.62	1.0	13.1	—



TEXT-FIG. 18.

No. 18 (*Case 4264*).—Female, factory worker; age 19 years. Moderately advanced; active. Sputum + — —, on admission, in course of treatment, and at present.

Onset 12 months ago with cough and expectoration; later marked temperature developed. Under treatment symptoms have improved, but physical signs have increased slightly.

	kg.
Height 157 cm. Theoretical normal weight	52.5
Present weight	62.0
Patient's idea of normal weight	54.5
Date of highest weight 3 months ago	63.5
" " lowest " 9 " "	51.5

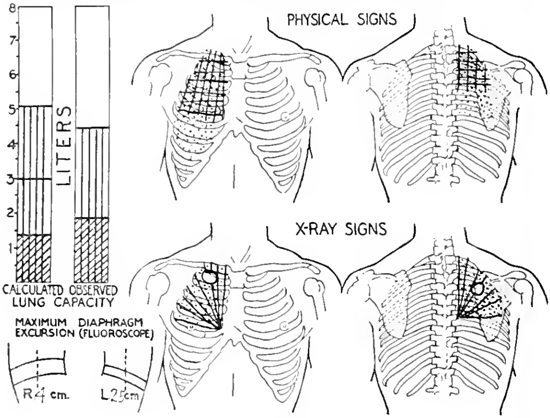
Treatment duration 5 months.

Physical Signs.—April 9, 1917. Right, slight impairment of resonance; breath sounds are a little increased in intensity; numerous fine and moist râles with cough to third rib anteriorly and fourth spine posteriorly; a few râles in this area on breathing without cough. Left, marked dullness; breath sounds are diminished in intensity; râles on breathing, but much increased in number by cough, extending to fourth rib anteriorly and sixth spine posteriorly.

X-Ray Signs.—April 7, 1917. Right apex clear; first, second, and third interspaces slightly spotted and striated. Left apex and first and second interspaces very dense; third interspace less dense. Mediastinal contents to the left. Right lung area increased.

No. 19 (CASE 3908)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT:POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	18.5	17.3	25.3	8.11	—	—	30.1
MAX INSP.	18.5	18.7	26.6	9.20	4.4	47.8	—
MAX EXP.	18.5	16.2	24.8	7.43	1.9	24.5	—



TEXT-FIG. 19.

No. 19 (Case 3908).—Female, factory inspector; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 25 months ago with malaise, slight loss of weight, slight cough, and slight temperature. Aphonia for last 10 months (tuberculous laryngitis). Under sanatorium rest temperature has become normal; lung lesion has progressed slightly.

	kg.
Height 173 cm. Theoretical normal weight	64.0
Present weight	70.0
Patient's idea of normal weight	60.0
Date of highest weight 14 months ago	77.5
" " lowest " 2 " "	69.5

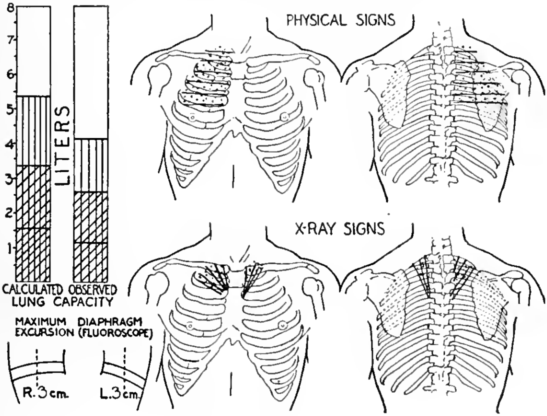
Treatment duration 14 months.

Physical Signs.—April 9, 1917. Right, dull to fifth interspace; harsh breathing; numerous very moist râles without cough through the lung anteriorly, and to the seventh spine posteriorly. Left lung seems normal.

X-Ray Signs.—April 7, 1917. Right lung moderately densely spotted and striated to the fourth interspace; cavity under the clavicle 5 cm. in diameter. Left lung clear. Mediastinal contents very markedly to the right. Left lung area greatly increased.

No. 20 (CASE 4103)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP / CHEST VOL.	RATIO 100 X VITAL CAP / CHEST VOL.
	STERNUM	ANT-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm	liters	liters		
REST	18.6	17.8	27.3	9.05	2.60	28.7	31.5
MAX.INSP.	18.6	18.5	28.5	9.80	4.15	42.3	——
MAX.EXP.	18.6	16.6	26.2	8.08	1.20	14.9	——



TEXT-FIG. 20.

No. 20 (*Case 4103*).—Female, cotton mill worker; age 25 years. Moderately advanced; active. Sputum + + −, on admission, in course of treatment, and at present.

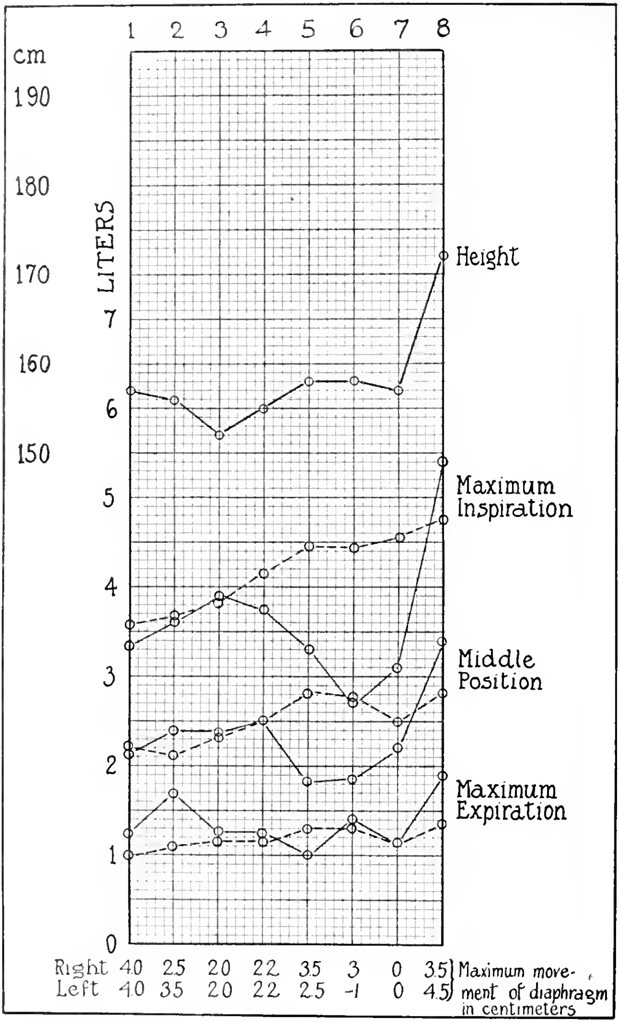
Onset 14 months ago with malaise, loss of weight, cough, and hemoptysis. Under sanatorium treatment the symptoms almost disappeared, and the signs are greatly diminished in number.

	kg.
Height 170 cm. Theoretical normal weight	62.5
Present weight	76.0
Patient's idea of normal weight	68.0
Date of highest weight 3 months ago	79.0
" " lowest " 15 " "	61 0

Treatment duration 9 months.

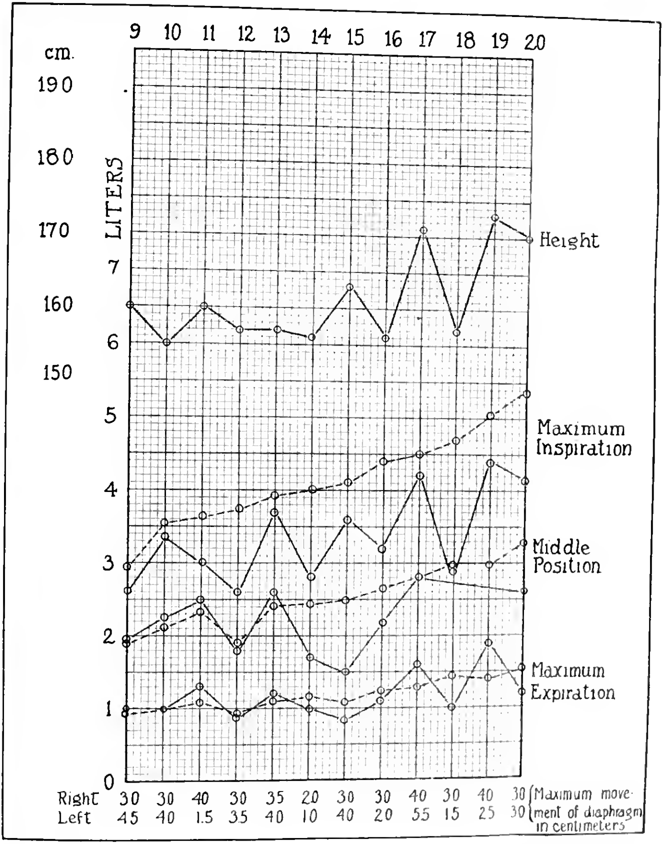
Physical Signs.—April 9, 1917. Right, moderate impairment of resonance; breath sounds increased in intensity and medium moist râles from apex to fourth rib anteriorly and to sixth spine posteriorly with aid of cough. Left lung seems normal.

X-Ray Signs.—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Left apex slightly infiltrated. Mediastinal contents normal.

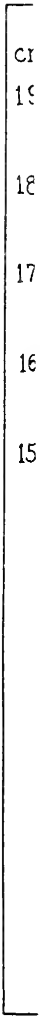


The numbers below indicate the maximum excursion of the right and left diaphragm. The numbers above the chart refer to the individual diagrams and descriptions.

TEXT-FIG. 21. Lung volumes in women with incipient pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



TEXT-FIG. 22. Lung volumes in women with moderately advanced and advanced (Nos. 9 and 19) pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



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THE SOLVENT ACTION OF ANTISEPTICS ON NECROTIC TISSUE.

By HERBERT D. TAYLOR, M.D., and J. HAROLD AUSTIN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATE 5.

(Received for publication, October 1, 1917.)

The recent interest in the chemical sterilization of wounds has led to the introduction of numerous new antiseptics, each of which has in turn been advocated because of some advantage, real or apparent. For many of these compounds, claims have been made which are not always confirmed by carefully controlled experiments. Carrel and Dehelly[1] emphasize that, for the removal of the necrotic tissue that remains after mechanical cleansing, Dakin's hypochlorite solution is the antiseptic of choice because of its solvent action on devitalized tissue, and Dakin and Dunham[2] also recognize the value of the hypochlorite solution for this purpose.

Dakin's solution was shown by Fiessinger and his coworkers[3] to have a disintegrating action on pus cells. Rous and Jones[4] have shown that intact leukocytes may protect virulent bacteria which they have phagocyted from the action of antiseptics, and that subsequently these bacteria may proliferate under suitable conditions. Dakin's solution, by its solvent action on these leukocytes, minimizes the danger of reinfection of the wound from this source. Because of this action on necrotic tissue, pus, and serum clot, Carrel and Dehelly recommend Dakin's hypochlorite solution for the sterili-

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 150, 192.

[2] Dakin, H. D., and Dunham, E. K., A handbook on antiseptics, New York, 1917, 14.

[3] Fiessinger, N., Moiroud, P., Guillaumin, C. O., and Vienne, G., *Ann. med.*, 1916, iii, 133.

[4] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.

zation of infected wounds.[5] Bashford[6] has demonstrated the ability of Dakin's solution in high dilution to erode the tissues of the tadpole's abdomen. He showed also that this occurred only after the circulation to the part had been interrupted for some time, due to the death of the organism.

As this erosive action of Dakin's solution is an important factor, the following experiments were planned to compare its solvent action with that of certain other chlorinated antiseptics. Fiessinger and his coworkers[3] concluded that the essential factor in the solvent action of the hypochlorites is their alkalinity. Our experiments were therefore designed to determine the importance of three factors: the alkalinity, the nature of the chlorinated antiseptic employed, and the chlorine concentration of the latter.

Method.

The solvent action of the various substances employed was tested by adding 50 cc. of each solution to 5 cc. of an emulsion of macerated liver tissue in a 100 cc. bottle. The mixture was thoroughly shaken every half hour for 2 hours. A 15 cc. portion was then removed to a centrifuge tube and in each case centrifuged at the same high speed for 5 minutes. The volume in cubic centimeters of the sediment thrown down was measured. The solvent action was shown by diminution of the amount of sediment compared with that obtained from inert solutions such as water or normal saline solution.

The liver emulsion was prepared in Experiment 1 from rabbit liver, in the other experiments from cat liver. In Experiments 1, 2, 3, and 4 the liver was purposely infected by handling, placed in the incubator at 37°C. until thoroughly necrotic, cut into small pieces, suspended in saline solution, shaken in a bottle with broken glass to emulsify it, and strained through a single layer of gauze. In Experiment 5 cat liver was similarly emulsified and used after 12 hours' preservation in the ice box. The solutions employed were prepared as follows:

[5] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 109.

[6] Bashford, E. F., *Lancet*, 1917, cxciii, 595.

Control Solutions.—Neutral: water and normal saline solution. Weakly alkaline: a solution of sodium carbonate, 1 gm., and sodium bicarbonate, 17 gm. per liter of water; this solution has approximately the alkalinity of a properly prepared Dakin's solution. Strongly alkaline: 0.1 N sodium hydroxide.

Chloramine-T Solutions.—Chloramine-T solutions were prepared by dissolving the required amount of chlorazene[7] in the appropriate control solution to obtain neutral, weakly alkaline, or strongly alkaline chloramine solutions.

Hypochlorite Solutions.—Weakly alkaline: ordinary Dakin's solution prepared either from bleaching powder or by the action of liquid chlorine on sodium carbonate solution, in either case with careful control of the degree of alkalinity as well as of the hypochlorite content. Neutral: chlorine gas passed through sodium carbonate solution, 28 gm. per liter, until the solution just ceased to give a flash of pink upon the addition of alcoholic solution of phenolphthalein, 1 per cent; the hypochlorite content was determined by titration, and the solution diluted with water to the desired strength. This solution was always prepared immediately before use, as a considerable proportion of the total hypochlorite is present as hypochlorous acid, and the decomposition of the solution is very rapid (Cullen and Austin[8]). Strongly alkaline: A double strength neutral solution of hypochlorite was prepared as just described and immediately added to an equal volume of 0.2 N sodium hydroxide solution.

Chlorinated Oils.—Paraffin oil and eucalyptol were mixed in equal parts, and the same oils chlorinated according to Dakin's method.[9]

Dichloramine-T.—A 15 per cent solution was made in chlorinated eucalyptol and then mixed with an equal volume of chlorinated paraffin oil. In the experiments in which the oils were used (Experiment 1, Tubes 5, 6, and 7; Experiment 2, Tubes 4, 5, and 6; Experiment 3, Tube 9), except in Experiment 3, Tube 10, 5 cc. of liver emulsion were added to 50 cc. of water or of one of the control solutions, and 15 cc. of the oil used were superimposed upon this mixture; the mixture was well shaken every half hour with a rotary motion.

[7] Prepared by the Abbott Laboratories, New York.

[8] Personal communication.

[9] Dakin, H. D., *Brit. Med. J.*, 1915, ii, 318.

At the end of 2 hours, after again being shaken, the oil was allowed to separate from the aqueous suspension and 15 cc. portions were immediately removed from the latter for centrifuging. In Tube 10 of Experiment 3, the 5 cc. of liver emulsion were introduced into 50 cc. of the oil without water and shaken continuously for 2 hours.

The sodium hypochlorite equivalent of the hypochlorite and other chloramine solutions was determined by the addition of potassium iodide solution and glacial acetic acid to 10 cc. samples and titration of the iodine liberated with 0.1 N sodium thiosulfate.

RESULTS.

In Experiment 1, Table I, Solutions 8 and 9 being taken as controls, no solvent effect was noted after the action of chloramine-T solution or of the chlorinated oils, with or without dichloramine-T. Dakin's solution, on the other hand, had a marked solvent action, which was apparent upon inspection of the bottles, even in the first 15 minutes. This was accompanied by pronounced bleaching of the emulsion. A still more rapid and marked action, similar in character, was obtained from the strongly alkaline hypochlorite. Figs. 1 and 2 show the results of this experiment at the end of 2 hours. The reaction of the

TABLE I.

Experiment 1.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral chloramine-T, 0.5 per cent, equivalent to sodium hypochlorite, 0.12 per cent	0.30
2	Neutral chloramine-T, 0.2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.25
3	Weakly alkaline hypochlorite, 0.5 per cent (Dakin's solution)	0.05
4	Strongly " " 0.5 " " " "	0.02
5	Water + paraffin oil and eucalyptol.*	0.27
6	" + chlorinated paraffin oil and eucalyptol.*	0.28
7	" + " " " " " + dichloramine-T, 7.5 per cent	0.27
8	Salt solution	0.28
9	Water	0.27

* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil.

solutions used, however, varied, as well as the nature of the antiseptic substance. Experiment 2 was therefore performed with solutions of approximately the same reaction (Table II).

The results of this experiment confirm those observed in Experiment 1. Chloramine-T and dichloramine-T were without solvent action, whereas Dakin's hypochlorite gave marked solution. In Experiment 3, Table III, the effect of diminishing the concentration of the antiseptic in a solution of the same reaction was tested. Both weakly alkaline and strongly alkaline solutions were employed.

TABLE II.

Experiment 2.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Weakly alkaline carbonate-bicarbonate solution....................	1.27
2	" " " " + chloramine-T, 2 per cent..	1.24
3	Weakly alkaline hypochlorite, stock Dakin's solution (sodium hypochlorite, 0.5 per cent)..	0.08
4	Weakly alkaline carbonate-bicarbonate solution + paraffin oil and eucalyptol.*...	1.23
5	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol.*..	1.24
6	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.........	1.32

* 5 cc. of liver emulsion in 50 cc. of carbonate-bicarbonate solution, overlaid with 15 cc. of oil.

This experiment showed a loss of the solvent action in weakly alkaline hypochlorite solutions, occurring suddenly between 0.2 and 0.3 per cent sodium hypochlorite concentration. In the strongly alkaline solutions the solvent action was marked, even at the lowest hypochlorite concentration employed. This experiment, taken in conjunction with the well known rapid drop in the sodium hypochlorite titer of Dakin's solution in contact with tissues, indicates that any solvent action resulting from its application clinically may be expected to occur in the first few minutes and emphasizes the importance of frequent flushing of wounds with the solution.

In order to distinguish between the effects of alkalinity and of hypochlorite concentration, Experiment 4 was performed (Table IV). The neutral hypochlorite solutions were prepared as described above. For the neutral control, saline solution was employed. The weakly alkaline hypochlorite solutions in the column headed "Weakly alkaline due to hypochlorite" were prepared as already described, except that the chlorine gas was passed into sodium hydroxide solution instead of into sodium carbonate, thus producing a solution of which

TABLE III.

Experiment 3.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Water..........	0.25
2	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent......	0.22
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)....	0 01
4	" " " " " 0.4 " "	0.01
5	" " " " " 0.3 " "	0.02
6	" " " " " 0.2 " "	0.22
7	Strongly " " " " 0.5 " "	Tr.
8	" " " " " 0.2 " "	0.08
9	Water + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*........	0 25
10	Chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent, without water.†	0 22

* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil. Shaken continuously for 2 hours.

† 5 cc. of liver emulsion in 50 cc. of oil. Shaken continuously for 2 hours.

the alkalinity was due almost entirely to sodium hypochlorite, and which was without buffer substances. The hypochlorite solution in the column headed "Weakly alkaline due to carbonate-bicarbonate" and the strongly alkaline solution were prepared exactly as described above. Table IV shows that in the control solutions solvent action occurred only in the strongly alkaline solution. A somewhat more marked solvent action was obtained when hypochlorite, even in the low concentration of 0.1 per cent, was added to the alkali. No solvent action was obtained in the weakly alkaline control, as compared

with the neutral control of normal saline solution. When hypochlorite was added to the weakly alkaline carbonate-bicarbonate solution, or when a weakly alkaline solution of sodium hypochlorite without carbonates was prepared, no solvent action was present at a concentration of 0.1 per cent, but it was marked at a concentration of 0.2 per cent. The change in solvent action resulting from small variations in hypochlorite concentration at about 0.2 per cent was striking, confirming the results of Experiment 3. The absence of solvent action of Dakin's solution below a hypochlorite concentration of 1:500 contrasts sharply with the marked bactericidal action of the same solution in serum to 1:1,500, and in water to 1:500,000 on *Staphylococcus aureus*.[10] In neutral solution at a hypochlorite concentration of 0.2 per cent, solvent action was very slight. At 0.3 per cent it was moderate, and at 0.5 per cent marked.

TABLE IV.

Experiment 4.

Tube No.	Hypochlorite concentration of solutions.	Reaction of solutions.			
		Neutral.	Weakly alkaline.		Strongly alkaline.
			Due to carbonate-bicarbonate.	Due to hypochlorite.	
		Sediment.	Sediment.	Sediment.	Sediment.
	per cent	*cc.*	*cc.*	*cc.*	*cc.*
1	0.5	0.01	0.01	0.01	Tr.
2	0.3	0.04	0.01	0.01	"
3	0.2	0.10	0.01	0.01	"
4	0.1	0.14	0.13	0.12	"
5	Control.	0.12	0.12		0.04

Experiment 5 serves as a final control of the solvent action of alkali alone, of chloramine-T added to neutral, weakly alkaline, and strongly alkaline solutions, and of hypochlorite solutions of the three grades of alkalinity. It is clear that chloramine-T, even in a 2 per cent solution, has no solvent action except that due to the alkalinity of the solution in which it is dissolved, and that therefore it is without this

[10] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

action in the grade of alkalinity permissible for clinical use. On the other hand, 0.5 per cent neutral sodium hypochlorite-hypochlorous acid solution (Tube 7) has a marked solvent effect, which must be attributed to the action of the chlorine unaided by alkali. Fig. 3 shows the results of Experiment 5 (Table V) at the end of 2 hours.

TABLE V.

Experiment 5.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral control (salt solution)	0.25
2	Weakly alkaline control (carbonate-bicarbonate)	0.24
3	Strongly " " (0.1 N sodium hydroxide)	Tr.
4	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.26
5	Weakly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.24
6	Strongly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	Tr.
7	Neutral hypochlorite (sodium hypochlorite, 0.5 per cent)	"
8	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's))	"
9	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)	"

Experiments upon leukocytes, erythrocytes, and plasma clot in the various solutions employed in the five experiments described gave results practically identical with those obtained with liver emulsion. When, however, discs of blood clot were employed, solvent action could not be demonstrated except, possibly, to a slight degree in the strongly alkaline solutions. Blood clot is the most resistant of the substances studied against the solvent action of the solutions used.

DISCUSSION.

From the results recorded above, it seems justifiable to lay considerable stress on the relatively great solvent action of Dakin's hypochlorite solution as contrasted with the more recent and more stable chloramines of Dakin. It also seems probable that to its greater ability to dissolve necrotic tissue, plasma clot, and leukocytes

it owes its chief claim to preference over the chloramines in the treatment of infected wounds. Curves shown by Carrel and Dehelly[11] demonstrate the relative ease with which this solution will sterilize grossly infected wounds in the initial presence of much necrotic tissue and pus.

The results of our experiments show that the solvent action of Dakin's hypochlorite solution in the degree of alkalinity used clinically is due primarily to its hypochlorite content. The slight alkalinity of Dakin's solution, while in itself without solvent action, does, however, increase the effectiveness of the hypochlorite. We are compelled to differ, therefore, from Fiessinger and his coworkers,[3] who attributed this action of the hypochlorite solutions to their alkalinity. In our weakly alkaline solutions, the solvent action of the hypochlorite solution ceased abruptly at about 0.2 per cent sodium hypochlorite concentration. This phenomenon occurs at a lower hypochlorite concentration as the reaction of the solution becomes more alkaline and *vice versa*. Even in neutral solutions, marked solvent action occurs at a hypochlorite-hypochlorous acid concentration of 0.5 per cent. A solution the alkalinity of which is equal to 0.1 N sodium hydroxide exerts a solvent action in the absence of any other factor. Such a solution, however, is not available for clinical use because of its irritating properties.

Chloramine-T failed in these experiments to exhibit any solvent action not explicable as an effect of the alkalinity of the solution in which it was dissolved, and dichloramine-T was also wholly without solvent action. The results of our experimental studies do not, therefore, support the clinical observations of Dakin and his associates,[12] who assert that "the chlorin in dichloramin-T, as in the hypochlorites, has the power of dissolving dead tissues," or similar conclusions reached by Sweet,[13] who states: "The dichloramin-T also possesses to a marked degree the characteristic power of the chlorin solutions in aiding the digestion and removal of necrotic, sloughing

[11] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 162, 170.

[12] Dakin, H. D., Lee, W. E., Sweet, J. E., Hendrik, B. M., and Le Conte, R. G., *J. Am. Med. Assn.*, 1917, lxix, 30.

[13] Sweet, J. E., *J. Am. Med. Assn.*, 1917, lxix, 1076.

tissues. The new solution seems more effective in cleaning up sloughing tissue than the older chlorin compounds." It seems probable that the greater solvent action of hypochlorite solution, as contrasted with the chloramines, is related to the greater instability of the former. We have been unable to demonstrate a solvent action on blood clot from any of the solutions of a reaction available for clinical use.

CONCLUSIONS.

1. Dakin's hypochlorite solution has the power of dissolving necrotic tissue, pus, and plasma clot in the concentration and reaction used clinically.

2. Chloramine-T and dichloramine-T do not exhibit this action.

3. The solvent action of Dakin's hypochlorite solution of the degree of alkalinity used clinically is due primarily to its hypochlorite content, but its slight alkalinity, while in itself without solvent action, enhances the effectiveness of the hypochlorite.

4. In the degree of alkalinity used clinically, the solvent action of hypochlorite is absent below about 0.2 per cent sodium hypochlorite concentration.

5. The hypochlorite concentration at which the solvent action ceases is lower the more alkaline the solution, and *vice versa*.

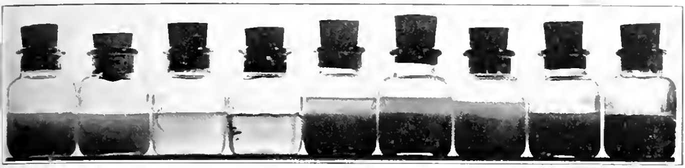
6. None of the antiseptics studied had demonstrable solvent action on blood clot.

EXPLANATION OF PLATE 5.

FIG. 1. Photograph of bottles in Experiment 1 (Table I).

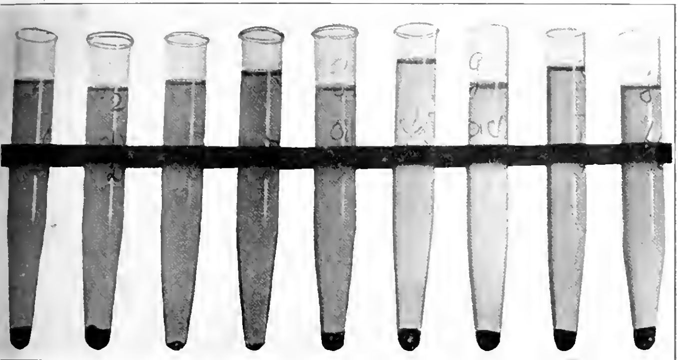
FIG. 2. Photograph of centrifuged samples from Experiment 1 (Table I).

FIG. 3. Photograph of centrifuged samples from Experiment 5 (Table V).



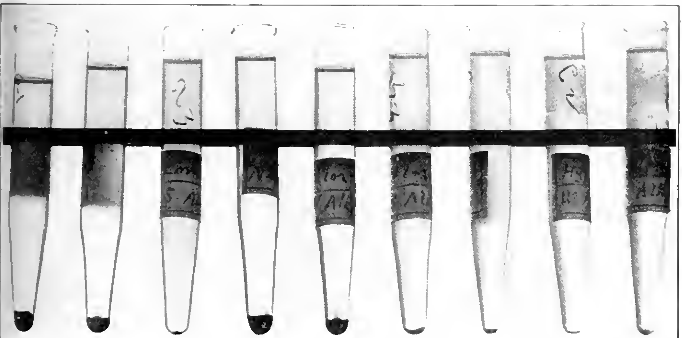
1 2 3 4 5 6 7 8 9

FIG. 1.



1 2 3 4 5 6 7 8 9

FIG. 2.



1 2 3 4 5 6 7 8 9

FIG. 3.

(Taylor and Austin: Solvent action of antiseptics.)

A NOTE ON THE PROGRESS OF CICATRIZATION OF WAR WOUNDS.

By THEODORE TUFFIER, M.D., and R. DESMARRES.

(*From Auxiliary Hospital 75, Paris, France.*)

Plate 6.

(Received for publication, March 26, 1917.)

The therapeutic effect of a physical or chemical agent on the cicatrization of wounds can be ascertained empirically from the rate of the process of cicatrization. To demonstrate this effect scientifically it is necessary first to determine exactly the normal evolution of a sterile wound,[1] that is the course of cicatrization in a given time; a unit of measure is thus obtained. When the normal progress of the cicatrization of a wound that is kept bacteriologically sterile is known, it becomes possible to study the changes taking place under physical or chemical influences.

This first question of a unit of measure was studied in Compiègne by du Noüy, under the direction of Dr. Carrel. We have worked according to du Noüy's formula, with the results here presented. All the work was done on wounded soldiers at Auxiliary Hospital 75.

To subject organic processes to a mathematical formula is always a hazardous procedure. However, we believe that by eliminating certain causes of error and adopting the controlled conditions of experimentation, results can be obtained which show the exact coincidence of mathematical forecasts with clinical evidence. This parallelism is constant and regular; if it becomes irregular, it is due to an error in therapeutic methods, thus furnishing a means of control of the care given to the patient.

The present work is the result of observations on twenty cases, in regard to the sterilization of the wounds as well as their rate of healing.

[1] By a sterile wound we mean bacterial or clinical sterilization; that is, with one or two cocci per field.

Cicatrization results from two processes, inodular contraction of connective tissue and epidermization. Contraction is in reality a mechanical action connected with the appearance of granulations on the surface of the wound.[2] We know from the work of du Noüy that a sterile surface wound heals in a period of time which can almost invariably be predicted by the following simple calculation:[3]

If S_1 and S_2 represent the surface area of a wound before and after t days, then

$$S_1 = S_2 \left[1 - i \left(t - \sqrt{T} \right) \right]$$

T representing the number of days elapsing since the first observation and i a constant characterizing each wound. This constant i, or the index, can be determined by means of the first two observations made, for example, at 4 days' interval.

i is expressed by the formula[4]

$$i = \frac{\frac{S_1 - S_2}{S_1}}{t + \sqrt{T}}$$

or for $t = T = 4$,

$$i = \frac{S_1 - S_2}{6S_1}$$

Du Noüy has shown that the index is clearly connected with the age of the patient and the initial surface of the wound; from a considerable number of observations, a chart[5] has been constructed which, on the assumption that the wound is sterile, makes it possible to determine the index *i a priori*, after only one measurement of the surface, when the age of the patient is known. The index varies approximately between 0.02 and 0.08.

The circumference of the wound or the epithelial edge can easily be traced on a sheet of sterilized cellophane; the surface is then measured in square centimeters with an Amsler planimeter. It is interesting also to observe at the same time the surface of the cicatrix; it can be seen almost invariably, during the days immediately preced-

[2] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[3] This information was kindly furnished by Captain Lecomte du Noüy, of Hospital 21, in Compiègne. For further detail see *J. Exp. Med.*, 1916, xxiv, 451, 461.

[4] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

[5] Text-fig. 1, du Noüy, *J. Exp. Med.*, 1916, xxiv, 463.

ing recovery, that the surface of the cicatrix broadens as if the contraction of tissue, having become unnecessary, ceased to occur.[2]

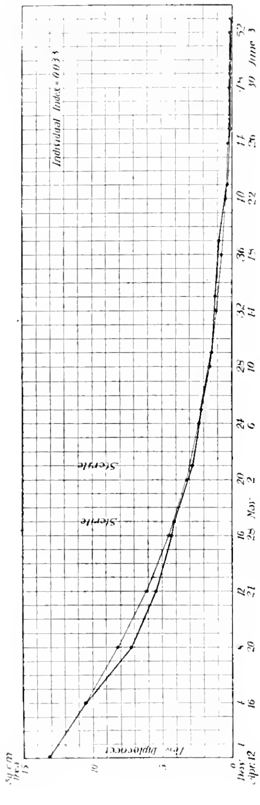
It is a simple matter to plot the calculated results or the direct observations in a curve with time as abscissæ and surface as ordinates. The curve resulting from the formula for $t = 4$, $T = 4, 8, 12, 16 \ldots.$ shows the normal course which the healing of a wound should follow. The observed surfaces give a second curve which follows the first closely unless there are irregularities to be explained by faulty dressings, reinfection, or illness of the patient. In the present experiments the theoretical curve is drawn with a light line, the curve from direct observation with a heavy line for the epithelial edge, and with a broken line for the cicatrix.

From the fact that the theoretical curve of cicatrization can be drawn in advance, with two measurements of the surface at 4 days' interval, it is seen that the date when cicatrization will be complete can be predicted, often long in advance (Text-figs. 1 and 2).

The technique is as follows: The wound is made clinically sterile by Carrel's method. We have then in the center a granulating surface (*S*, Text-fig. 3) surrounded by an epithelial border (*L,L*); beyond, at the periphery of the surface of the new epithelium, is a line of union of the normal skin and new epithelium (*C,C*). The wound closes by two processes: (1) contraction of the granulating tissue (inodular contraction); (2) epidermization of the granulating surface. These two processes have a relative efficacy for cicatrization, differing according to the wound.

The course of cicatrization is most easily studied in a wound the site of which is not constantly in motion (as the bend of a joint), or one which is extendible and is well nourished, not a scalp wound or one due to loss of a large part of the skull.

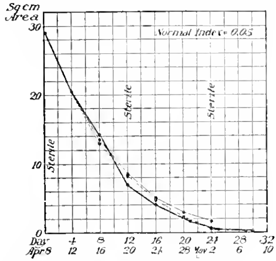
The operator, having washed his hands with alcohol, applies to the wound, which has previously been dried with a sterile compress, a thin sheet of cellophane sterilized in the autoclave at 120°C. for 1 hour. In order to preserve the pliability of the cellophane during sterilization it is well to enclose it in a glass tube in the bottom of which is placed a tampon soaked in glycerol diluted one-fourth with water; the cellophane rests on a tampon of non-absorbent cotton, and another closes the tube.



—— Observed curve.
—— Calculated curve.
—— Cicatrix (area of scar).

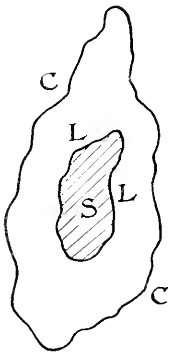
TEXT-FIG. 1. Experiment 1. Age 28 years. Superficial wound cicatrizing without incident, according to the formula.

Then with a dermographer's pencil, or, better, with pen and ink, as that allows a sharper and more exact line, a tracing is made of the



Curve corrected according to shape.

TEXT-FIG. 2. Experiment 2. Age 21 years. Long, narrow, superficial wound, cicatrizing without incident toward the date predicted. The corrected formula for this special kind of wound was employed in tracing the theoretical curve.[6]



TEXT-FIG. 3. Diagram of a wound, showing the granulating surface (S), surrounded by an epithelial border (L,L), and the line of union of the normal skin and new epithelium (C,C).

[6] For wounds of this sort du Noüy's corrected formula should be used:

$$S_n = S_{n-1}\left[1 - i\,(t + \sqrt{T})\right] - \frac{\sqrt{S_{n-1}}}{S_{n-1}}$$

See also du Noüy, *J. Exp. Med.*, 1917, xxv, 721.

epithelial edge and of the shape of the cicatrix. This is transferred to a sheet of ordinary paper, and on this are made the surface measurements with the planimeter.

Surface Wounds.

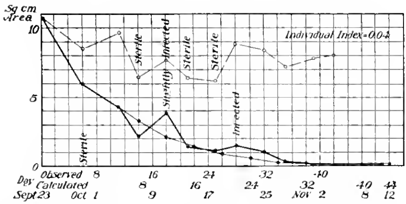
We have in this way studied a number of wounds treated with Dakin's solution by Carrel's method, keeping the bacterial flora regularly under observation both as to number and kind. Carrel's bacteriological curve was made for each wound.

Microscopic examinations, necessarily hasty, have no great value unless the results are positive, but the use of the curve sometimes points out a reinfection before it is observed, that is, before the microscope reveals it, as the observed curve deviates abruptly from the calculated curve,—which shows that cicatrization curves are not without value from the clinical point of view.

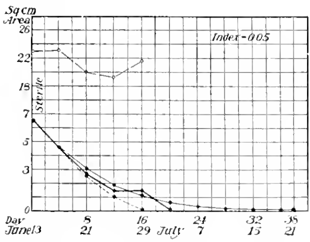
For wounds which are kept sterile the law holds, and cicatrization follows almost exactly the calculated curve (Text-figs. 1 and 2). If a wound previously sterile becomes accidentally infected and then becomes sterile again, the observed curve which separated from the calculated curve often rejoins it rapidly, and cicatrization is complete within a few days of the time predicted (Text-fig. 4). There seems to be a production of substances aiding cicatrization in the period preceding the infection. The substances are carried in the circulation but reinfection prevents their being used for epithelial cicatrization.

Cicatrization of certain wounds sometimes follows, at the beginning or later, a course more rapid than the calculated curve indicates. In such a case, if the wound is not a long, narrow one,[6] secondary ulcerations, in which we have never found bacteria, occur and restore the surface of the wound to what it would have been theoretically (Text-figs. 5 and 6). If infection entirely destroys epidermization, it is restored much more rapidly than the calculated curve indicates (Text-figs. 5 and 6). In Text-fig. 6 the observed curve oscillates about the calculated curve. It is difficult in this case to find a satisfactory explanation of this phenomenon; it can only be said that secondary ulcerations are related to the presence of bacteria in the deeper

parts of the wound. It is necessary in all such cases to know whether the bacteria are not retarding cicatrization. Certain cocci commonly observed allow normal cicatrization, but we have never found bacilli that allowed the same evolution of cicatrization.



TEXT-FIG. 4. Experiment 3. Surface wound of the right knee. The wound healed according to the law. Two infections in the course of cicatrization did not prevent the wound from healing in the calculated time.

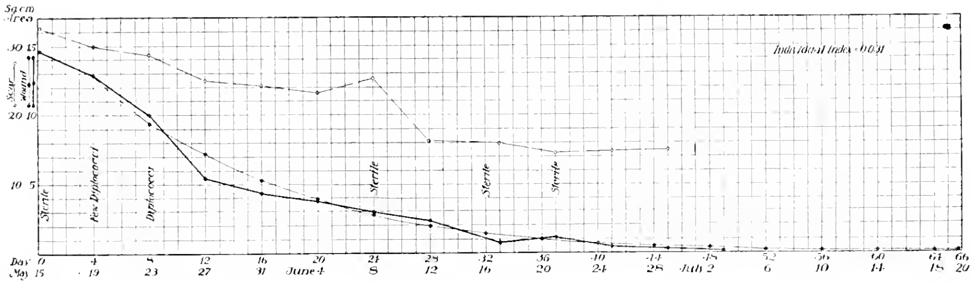


Curve corrected according to shape.

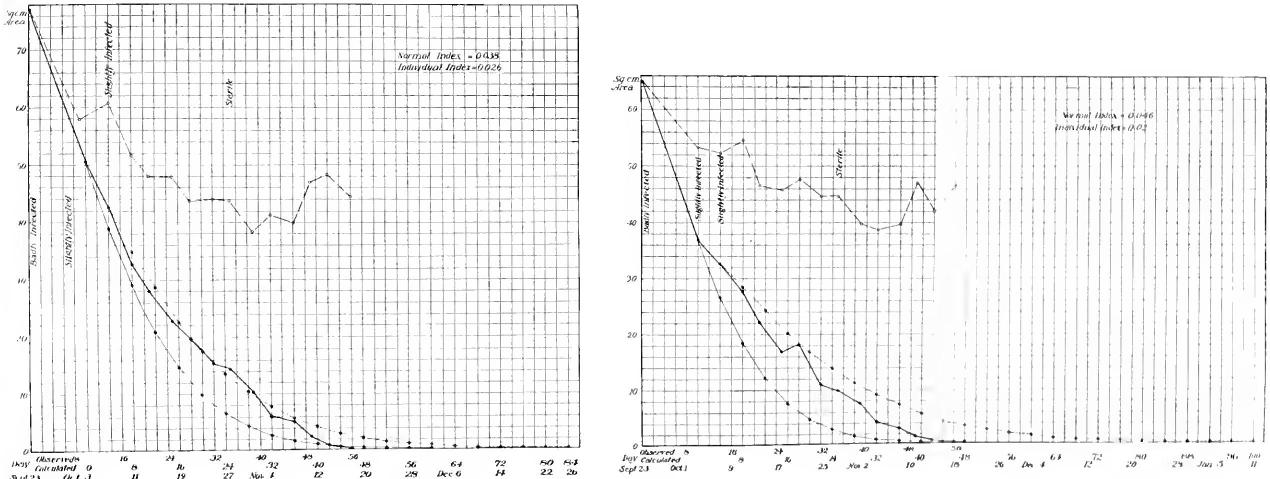
TEXT-FIG. 5. Experiment 4. Age 30 years. Superficial wound of the abdomen, in the iliac region. The wound was aseptic during the entire period of cicatrization. It healed at first more rapidly than the theoretical curve indicated, but more slowly than the curve from the corrected formula for long, narrow wounds.

From information subsequently received from the patient himself it was learned that the wound reopened and finally healed on July 19, as the curve had indicated.

Certain infective processes produce ossifying myositis or hospital gangrene, the first with an extension of the wound, which spreads out as a flower opens, the second with induration of tissue which will



TEXT-FIG. 6. Experiment 5. Age 31 years. Surface wound of the right leg. The observed curve oscillates about the calculated curve. When the real surface is smaller than the calculated surface, secondary ulcerations, in which no bacteria have been found, bring the observed surface back to a size very near the theoretical one. Hitherto no satisfactory explanation has been found for this phenomenon. See also Experiment 4.



a

b

Calculated curve with normal index.

Calculated curve with individual index.

TEXT-FIG. 7, *a* and *b*. Experiment 6. Age 21 years. Two parallel wounds in the back, (*a*) upper wound, (*b*) lower wound. The wounds were s rile during the period of cicatrization. The curves show the effect produced by contraction of tissue; each wound alone would have cicatrized according to the calculated curve, taking for the index the ures furnished by the chart,[6] for 50.35 and 36.85 sq. cm., and 21 years. The index calculated from the first two surface measurements gave a second theoretical curve, drawn with a dotted line. T real curve of cicatrization remains almost entirely between the two calculated curves, especially for the lower wound. From the time when contraction no longer occurred (the broken line toward the h day), cicatrization took place rapidly, overtaking the calculated curve.

not contract. Thus it is clinically demonstrated that the nature of the infecting organism can influence the rapidity of cicatrization.

We have observed that if the mechanical contraction of tissue is hindered, by adhesions for instance, cicatrization occurs more slowly than is indicated by the calculated curve. Thus, cicatrization of two wounds near each other can be restricted by contraction in opposite directions. An example of this is Experiment 6 (Fig. 1, Text-fig. 7, *a* and *b*), two long parallel wounds in the dorsal region, generally sterile, restricting each other. Examination of the curves shows that the cicatrization of each wound if it had been alone would have followed the light line (index determined by the chart[5]); in reality it more nearly followed the dotted line (index determined from two actual measurements), at a slower rate. But in the last days before healing was complete, contraction of the tissues ceased (see the broken line), cicatrization went on much more rapidly, and the observed curve caught up with and even passed the calculated curve.

This experiment is related to a second theoretical cicatrization curve which we have frequently worked out. A continually infected wound can cicatrize according to the law provided, first, that the infection is within certain limits, that is that the number of bacteria is at most 14 to 15 per field; and second, that the infection is from cocci or diplococci, and that streptococci or bacilli are found only exceptionally. A theoretical cicatrization curve can be drawn from an index obtained from the two surfaces first measured. The observed curve will follow the calculated curve, and one should keep as close as possible to this.

Furthermore, a second theoretical curve may be made with the index determined by the chart,[5] from the initial surface of the wound and the age of the patient. This, which would be the cicatrization curve if the wound were sterile, is the normal curve, and we should try to follow it in treating the wound. The observed curve will lie practically between the two calculated curves (Text-fig. 8, *a*).

It seemed possible that the two curves could be of use when it is understood that the calculated index is not equal to the index determined by the chart, the two curves thus drawn constituting two limits between which the observed curve should continually remain.

Deep Wounds.

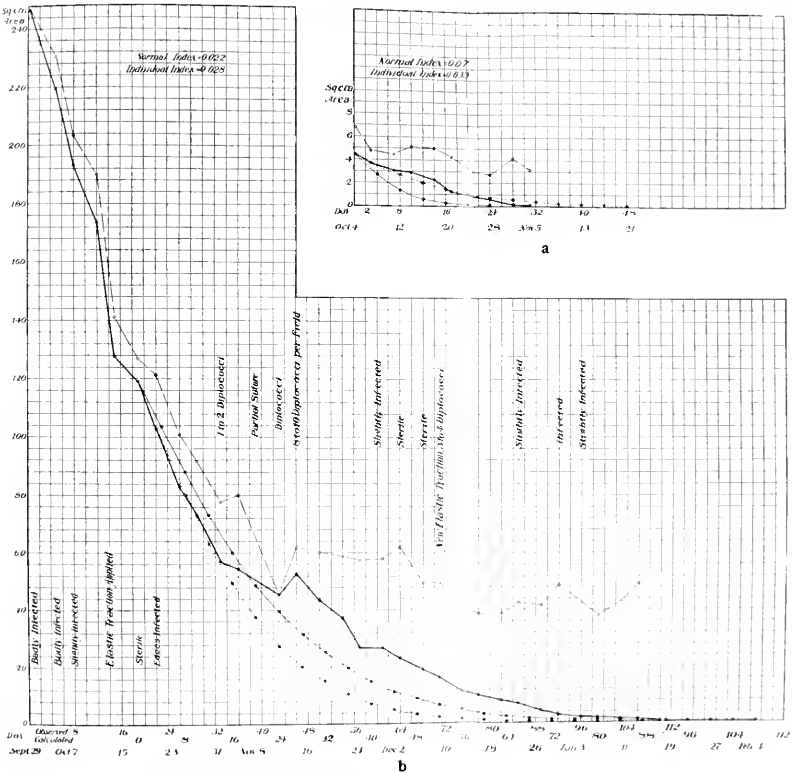
By an analogous procedure we have tried to follow the cicatrization of deep wounds. We first undertook to measure the volume of the wound by filling it with the fluid used for treating it, by means of a graduated pipette which could easily be sterilized. But the practical difficulty of placing the patient in a position in which the wound could be easily and thoroughly filled was so great, and the volume of the wound, which was often a cavity, was so variable, according to the patient's position, that we abandoned this procedure.

It seemed better to follow the indirect method of tracing on sterilized cellophane the successive outlines of the wound, as if we were dealing with a surface wound, and then to measure these areas.

By means of calculations identical with those which served for surface wounds, we have determined a theoretical cicatrization curve with an index calculated from the first two observed surfaces, or with an index determined from the chart. We have thus been able to show that a deep wound properly treated and sterilized, the edges of which are gradually drawn together at the proper time by adhesive plaster, with elastic strapping,[7] should cicatrize at least equally and often even more rapidly than a surface wound of the same contour (Text-figs. 9, *a* and *b* and 10, *a* and *b*). We have also observed that the gradual drawing together by means of the elastic traction is superior to the partial closure by suture intervening in the course of cicatrization (Text-fig. 8, *b*).

On October 13 the wound had been sterilized and the edges drawn together by an elastic traction; from that day cicatrization continued normally and steadily, and the observed curve lay between the two calculated curves (light line and dotted line). On November 7 the wound was partially sutured and decreased from 50 to 45 sq. cm., but presumably, from the general aspect of the curves, normal cicatrization would have decreased it to 35 or 36 sq. cm. After the operation the wound was reinfected and the surface extended to 52.75 sq. cm., decreasing again immediately, but at a much slower rate than was indi-

[7] Without the bandage a deep wound, at least one of small dimensions, seems to cicatrize according to a law analogous to that of the cicatrization of surface wounds, but more slowly.



Calculated curve with normal index.
Calculated curve with individual index.

TEXT-FIG. 8, *a* and *b*. Experiment 7. (*a*) Small wound. Example of cicatrization of a wound slightly but constantly infected. (*b*) Large deep wound. Example of a sterile wound whose edges have been drawn together by an elastic traction. An attempt at a partial suture was made, the action of which seems to have been injurious, if one considers that the observed curve deviates from the calculated curve. A new elastic traction placed December 9 hastened cicatrization; the observed curve tended to rejoin the calculated curves.

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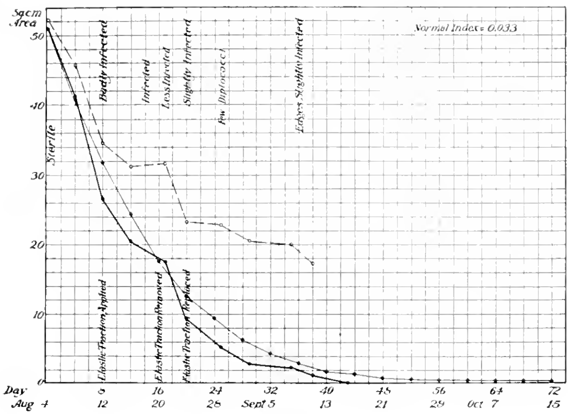
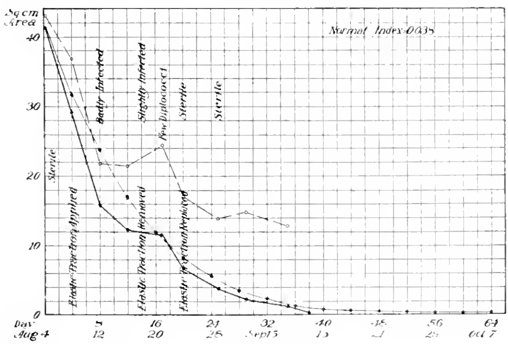


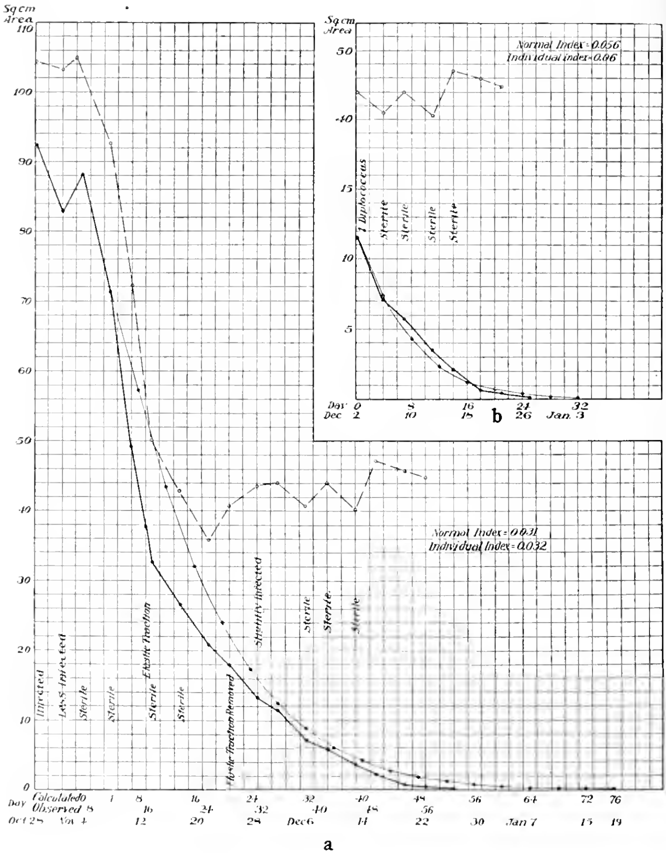
a



b

Calculated curve for superficial wound.

TEXT-FIG. 9, *a* and *b*. Experiment 8. Age 25 years. Deep hip wounds. The observed curves show the effect of the elastic traction on cicatrization, which took place rapidly in spite of the frequent presence of bacteria.



Calculated curve with normal index.

TEXT-FIG. 10, *a* and *b*. Experiment 9. Age 21 years. (*a*) Deep bullet wound in the lumbar region, treated with an elastic traction and cicatrizing more rapidly than a surface wound of the same contour. The smaller curve (*b*) was drawn after December 2, when the wound had become entirely superficial.

cated by the calculated curves, until the application of a second elastic traction again hastened the healing process. No conclusion can be drawn from this experiment, but it shows that in certain conditions in longitudinal wounds simply drawing the edges together gives rapid results.

SUMMARY.

Some hypothetical conclusions bearing on the evolution of cicatricial tissue can be suggested. The arterial circulation deposits in the wound chemical substances necessary for contraction of the wound and for epithelial proliferation. When the biologic process is not hindered by any special or severe bacterial infection this deposit is as regular as the circulation itself, and enables us to determine in advance the date of cicatrization. It even seems as though when the epidermization process is retarded by a slight infection the substances necessary for epidermization are stored up in the wound, and when the delay due to infection is removed the epithelium finds an accumulation of nutritive substances, and, so to speak, makes up the lost time.

Moreover, when an infection entirely or partially stops epidermization, we have observed (Experiments 3, 4, and 5) that after the infection has disappeared the progress of new epidermization is much more rapid than normally; it even passes the calculated curve. The infection apparently destroyed only the epithelium and left in the wound the chemical substances which activate epidermization.

The existence of these physical or chemical activating agents has been indicated again by two anatomical clinical facts. In treating a scalp wound in which there had been practically no epidermization for many months, we applied over the entire surface of the sterile wound dermo-epidermic grafts of fetal skin. After apparently taking, the grafts were absorbed and disappeared, but epidermization of the periphery of the wound, which hitherto had not progressed, took place abundantly, almost a hundred times as much as before.

We believe that by mathematical measurements we can solve the problem of the action of various organic fluids on the cicatrization of wounds.

EXPLANATION OF PLATE 6.

Fig. 1. Experiment 6. Age 21 years. Two parallel wounds in the back. Appearance of the wounds on October 6, 1916.

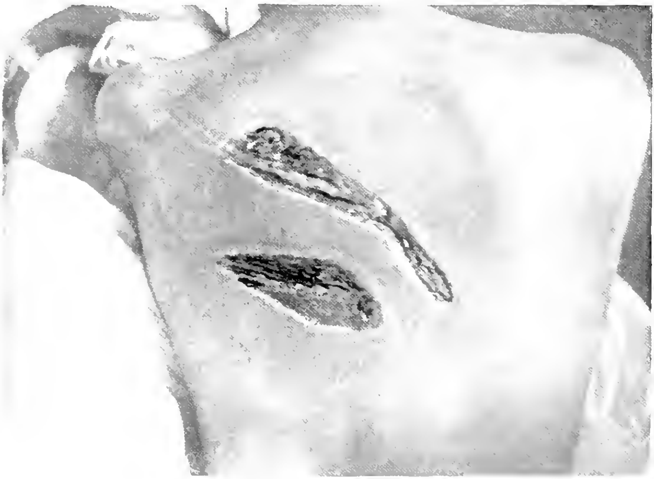


FIG. 1.

(Tuffier and Desmarres: Cicatr[illegible]ds.)

STUDIES OF OXYGEN IN THE VENOUS BLOOD.

II. Studies of the Oxygen Unsaturation in the Venous Blood of a Group of Patients with Circulatory Disturbances.

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(Received for publication, October 23, 1917.)

INTRODUCTION.

In a previous paper (1) a report was made of a series of determinations of the oxygen content of the venous blood from the vena mediana cubiti of twelve normal resting individuals. The difference between the oxygen in the venous blood and the total oxygen capacity of the hemoglobin (determined by Van Slyke's method (2) or Palmer's method (3)) was called the oxygen unsaturation of the venous blood. In this way the differences in hemoglobin concentration are eliminated.[1] It was found that the extent of oxygen unsaturation of the venous blood in normal individuals fell between 2.5 and 9 cc. per 100 cc. of blood. However, values above 8 volume per cent were only met with under special conditions; namely, when the blood was drawn in the morning immediately after the subject had been awakened from sleep. The blood was drawn from an arm vein without any stasis whatever, after the subject had rested for a half hour on a couch or bed. A sharp, not too pointed, needle was connected with a rubber tube to a glass pipette, 25 to 30 cm. long and $\frac{1}{2}$ cm. wide, which had a film of oxalate on the inside.[2] The blood (about 10 cc.) was sucked

[1] A determination of the oxygen in the venous blood without a simultaneous determination of the total oxygen capacity is just as incomplete as a determination of the urea in the blood or the nitrogen in the urine, without considering the intake.

[2] This was obtained by wetting the tube and rubber with a concentrated solution of oxalate and drying by an air current.

TABLE I.

Patients with Circulatory Disturbances Compensated at Rest.

Experimental.

Case No.	Determination No.	Age.	Bleeding.			Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a − v).	Pulse	Respirations.
			Date.	Hour.	Condition.*	Sample 1. Hour.	Sample 1. Result.	Sample 2 Hour.	Sample 2 Result.	Average (v).						
		yrs.	*1917*				*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
1	1	41	Feb. 3	11.00 a.m.	1 hr.	11.20	17.72	11.30	18.70	18.21	123	22.70	21.96, 22.34	4.49	98	18
2	2	56	May 22	10.30 a.m.	In bed.	11.35	12.98	11.55	13.50	13.24	100	18.50		5.26	80	22
3	3	47	Feb. 12	4.00 p.m.	5 min.	4.15	15.30	4.25	15.14	15.22	100	18.50	18.74, 18.00	3.28	84	24
	4		May 6	2.30 "	1 hr.	2.50	14.24	3.15	14.26	14.25			16.90, 17.10	2.75	100	22
4	5	29	May 8	2.10 p.m.	In bed.	2.40	5.44	3.00	5.42	5.43	44.5	8.23		2.80	84	22
5	6	49	May 22	9.20 a.m.	In bed.	10.50	9.19	11.08	9.35	9.27	92	17.02		7.75	80	22
	7		June 21	11.00 "	" "	11.45	12.24	12.00	12.24	12.24	89.5	16.54		4.30	80	20
	8		" 27	9.30 "	¾ hr.	10.30	11.88		11.46	11.67	88.5	16.37		4.70		
	9		" 30	10.20 "	¾ "	10.35	10.89	10.55	10.93	10.91	88.5	16.37		5.46	80	20
6	10	22	June 26	9.50 a.m.	½ hr.	10.50	11.78	11.20	12.04	11.91	103	19.06		7.15	82	20
7	11	25	Apr. 18	12 n.	In bed.	12.40	13.52	12.55	13.60	13.56	111	20.53		6.97	76	24
8	12	37	Feb. 12	12 n.	In bed.	12.15	15.30			15.30	111	20.53		5.23	75	18
9	13	31	Feb. 17	3.00 p.m.	In bed.	3.50	17.15	4.15	16.90	17.03	114	21.10		4.07	67	26
	14		Mar. 8	10.00 a.m.	" "	11.10	14.62	11.40	14.42	14.52	106	19.60		5.08	90	22
10	15	57	June 27	12.30 p.m.	½ hr.	2.10	12.89	2.30	12.47	12.68	105	19.44		6.76	96	28
11	16	25	May 31	3.00 p.m.	¾ hr.	4.00	11.65			11.65	89	16.46		4.81	56	28
12	17	18	Feb. 28	10.30 a.m.	In bed.	10.55	6.72	11.10	6.75	6.74	71.4	13.20		6.46	80	22

Clinical.

Case No.	Determination No.	Oxygen unsaturation (a − v).	Diagnosis and clinical notes.
		vol. per cent	
1	1	4.49	Arteriosclerosis; hypertensio (blood pressure 160–96); hypertrophy of heart; shortness of breath on exertion.
2	2	5.26	Arteriosclerosis; hypertension; hypertrophy of heart (blood pressure 296–130); some moist râles in both lungs.
3	3 4	3.28 2.75	Chronic interstitial nephritis; hypertension (blood pressure 180–130); hypertrophy of heart; shortness of breath on exertion.
4	5	2.80	Chronic interstitial nephritis; chronic uremia; hypertension (blood pressure 195–133); hypertrophy of heart; edema; ascites; stasis in lungs (edema); urea retention in blood.
5	6	7.75	Chronic nephritis; hypertension; cardiac hypertrophy (he was never incompensated at rest. He had shortness of breath on slight exercise). May 22. A few râles at base of lungs; no dullness. Blood pressure 218–126.
	7	4.30	June 21. No râles. Blood pressure 214–108.
	8	4.70	" 27. Pains behind sternum. No râles. Blood pressure 220–112.
	9	5.46	" 30. No râles.
6	10	7.15	Aortic insufficiency; hypertrophy of heart (blood pressure 300–90).
7	11	6.97	Mitral stenosis; auricular fibrillation.
8	12	5.23	Mitral stenosis and insufficiency; auricular fibrillation.
9	13 14	4.07 5.08	Mitral insufficiency; auricular fibrillation.
10	15	6.76	Auricular fibrillation; chronic myocarditis.
11	16	4.81	Chronic myocarditis. Pulse regular.
12	17	6.46	Pericarditis; some râles at bases of lungs.

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

TABLE II.

Patients with Incompensated Heart Failure.

Experimental.

Case No	Determination No.	Age.	Bleeding. Date.	Bleeding. Hour.	Bleeding. Condition.	Oxygen content of venous blood. Sample 1. Hour.	Sample 1. Result.	Sample 2. Hour.	Sample 2. Result.	Average (v).	Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a − v).	Pulse.	Respirations
		yrs.	*1917*				*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
13	18	30	Feb. 10	11.00 a.m.	1½ hr.	11 20	6 56	11.35	6 50	6.53	93	17.18	{16.65 16.69	10.14		
	19		" 19	10.05 "	In bed.	10.30	6.67	1.45	6.58	6.63	100	18 50		11.87	62	22
14	20	57	Feb. 9	3.10 p.m.	In bed.	3.30	8.60	3.50	8 84	8.72			{22.52 22.46	13.76	96	36
	21		" 19	10.00 a.m.	" "	10.10	5.93	10.50	5.79	5.86	112	20.71		14.85	86	32
	22		Apr. 27	2.40 p.m.	" "	2.45	12.39	3.10	12.55	12.47	120	22.20		9.73	86	32
	23		June 26	10.00 a.m.	" "	10.10	7.16	10 40	7.62	7.39	106	19.61		12.22	77	24
15	24	64	Feb. 21	11.10 a.m.	In bed.	11.20	12.12	11.30	12.05	12.07	119	22.00		9.91	36	20
	25		Mar. 24	12 n.	" "	2.30	7.68	2.50	7.66	7.67	117	21.65		13.98	28	20
	26		Apr. 21	11.00 a.m.	" "	2.10	10.48	2.30	10.46	10.47	110	20.36		9.89	32	24

Clinical.

Case No	Determination No.	Oxygen unsaturation (a – v).	Diagnosis and clinical notes.
		vol. per cent	
13	18	10.14	Mitral stenosis and insufficiency; auricular fibrillation; adherent pericardium.
	19	11.87	Râles at base of lungs; cyanosis of face; distention of veins in neck; enlargement of liver; ascites; low diuresis.
14	20	13.76	Mitral insufficiency; arteriosclerosis; hypertrophy of heart.
			Feb. 9. Moist râles in both lungs; severe cyanosis; enlargement of liver; severe edema of legs.
	21	14.85	" 19. Same condition.
	22	9.73	Apr. 27. Has improved. Very slight cyanosis; no edema; marked enlargement of liver. Still some râles in lungs.
	23	12.22	June 26. Slight edema of legs; slight cyanosis. Still râles at base of lungs.
15	24	9 91	Heart block; syphilis of heart.
			Feb. 21. Slight cyanosis of lips; slight jaundice of scleræ and skin; râles in lungs; no edema; great enlargement of liver.
	25	13.98	Mar. 24. Same condition.
	26	9.89	Apr. 21. " "

TABLE III.

A Patient with Incompensated Heart Failure. Oxygen Determined by Barcroft's Method.

Experimental.

Case No.	Determination No.	Age.	Bleeding. Date.	Bleeding. Condition.	Oxygen content of venous blood. Sample 1.	Sample 2.	Average (v).	Total oxygen capacity (Barcroft's method (a)).	Oxygen unsaturation (a − v).	Pulse.	Respirations.
		yrs.	*1916–17*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
	27	17	Oct. 25	In bed.	2 40		2 40	15.30	12.90		
	28		Nov. 20	" "	2 48		2 48	17 20	14.72	120	40
16	29		" 21	" "	3.36	1.36	2 36	17 56	15.20	122	40
	30		" 22	" "	2.40		2 40	17 56	15.16	124	44
	31		Jan. 12	" "	6 86		6 86	17 20	10 34	120	30

Clinical.

Case No.	Determination No.	Oxygen unsaturation (a − v).	Diagnosis and clinical notes.
		vol. per cent	
	27	12 90	Mitral and aortic insufficiency. Oct. 25, 1916. Moderate cyanosis; few râles at base of lungs.
	28	14 72	Nov. 20. Lungs clear.
16	29	15 20	" 21. Cyanosis.
	30	15 16	" 22. Enlargement of liver.
	31	10 34	Jan. 12, 1917. Less cyanosis; no râles; slight enlargement of liver.

up in the pipette, from which it was discharged into a cylinder, 2 cm. in diameter, below a layer (2 cm.) of mineral oil to prevent oxidation. The last (upper) 1 or 2 cc. of blood, a part of which had been oxidized, were put in a separate dish and used for a Palmer determination of the hemoglobin. Samples of blood for a Van Slyke determination were taken from the cylinder after careful stirring.[3]

Observations on Patients.

This paper is a report of a preliminary investigation on the oxygen unsaturation in patients with circulatory disturbances. Thirty-

[3] For further technical details see Paper I of this series (1).

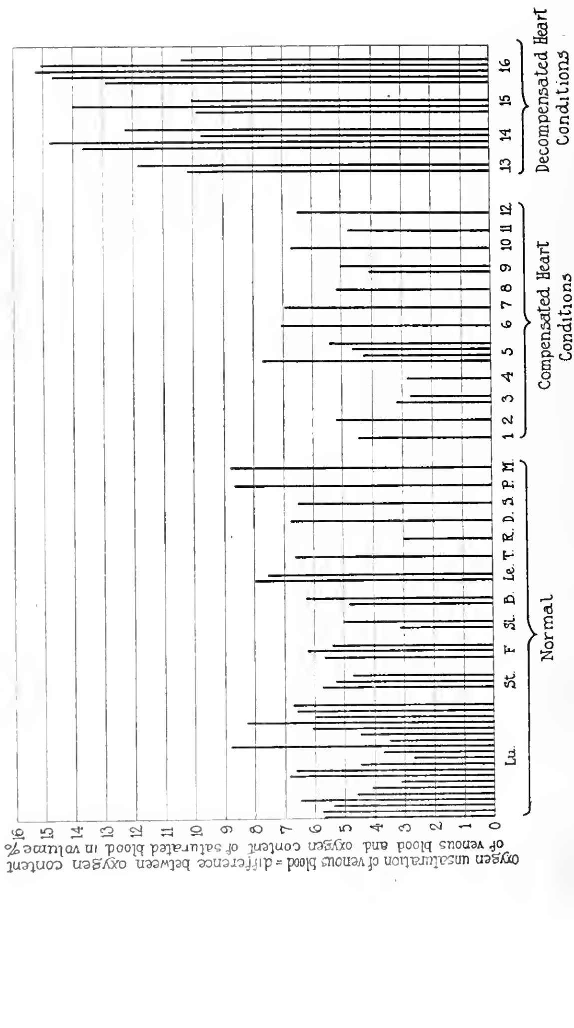
one determinations were made on sixteen patients with different kinds of circulatory disturbances. The procedure in preparing the patients and the technique in drawing the blood have been exactly as described in the first paper. Van Slyke's method (2) has been used for the determination of the oxygen in all but one case (No. 16 in Table III) where Barcroft's method has been used.

The patients have been divided into two groups according to the condition of the circulation. Results and clinical notes from patients with clinically compensated heart lesions have been tabulated in Table I. The data from the cases with incompensated circulatory conditions are collected in Tables II and III. As in Paper I, we have tabulated both the oxygen content of the venous blood samples and the extent of the oxygen unsaturation.

One case (No. 4, Table I) is particularly worth mentioning, because it shows the importance of using the oxygen unsaturation and not the absolute value of the venous oxygen alone in comparing the results from different individuals. This patient was brought to the hospital in a very sick condition. He was suffering from dyspnea and palpitation and had ascites and severe edema of the legs. The oxygen in his venous blood was 5.43 volume per cent, which was much lower than any figure obtained on normal individuals, and even lower than most of the figures obtained from patients with incompensated heart disturbances. The total oxygen-combining power of his blood was very low, however, 8.23 volume per cent, instead of the usual average 18.50 volume per cent. The oxygen unsaturation of his venous blood is therefore not only within normal limits but even rather low. The clinical diagnosis was chronic nephritis, chronic uremia, hypertension, hypertrophy of the heart, anemia, and urea retention. The edema was considered to be entirely of nephritic origin. Therefore, in respect to both the clinical picture and the findings in the blood, he is to be grouped in Table I, with patients free from incompensated circulatory disturbances. On the other hand, we may find a high figure for the venous oxygen combined with a high extent of unsaturation (No. 13, Table II) on account of a high content of hemoglobin.

Text-fig. 1 is a diagrammatic representation of the figures for the oxygen unsaturation in normal individuals[4] and in the patients re-

[4] Reported in Paper I (1).



TEXT-FIG. 1. Oxygen unsaturation of venous blood in a group of normal subjects[4] and of patients reported in the present paper.

ported in the present paper. It will be seen that the upper limit for the unsaturation in normal individuals (9 volume per cent) is not exceeded by any figure obtained from patients belonging to the clinically compensated group. The figures are all distributed in the same haphazard manner over the area between 2.5 and 9 volume per cent.

The figures from the incompensated group show quite another picture. They are all above the upper normal limit, lying between 9.7 and 15.2 volume per cent.

No attempt has been made to subdivide the cases according to the anatomical or physiological form of the heart lesion. There may be a difference, but the cases and the number of determinations in each case are much too few to allow any such attempt. The only statement which can be made is that the oxygen unsaturation of the venous blood from patients with clinically compensated heart conditions has been found within the normal limits, whereas the oxygen unsaturation in some patients with incompensated heart lesions has been above the upper normal limit. It is probably not even justifiable to make this a general statement. It seems probable that patients with non-stationary conditions might fall outside this rule. We might suppose that a patient with an incompensated, but improving heart lesion could show normal figures. On the other hand, a patient with a heart lesion where incompensation is developing might possibly show increased oxygen unsaturation before the ordinary clinical symptoms had developed. If that should be true, the determination of the oxygen unsaturation would be of great clinical significance. Series of determinations on a single patient might throw light on this question.

DISCUSSION.

There is extensive clinical and experimental evidence for assuming that the condition of the heart has a certain influence on the extent of oxygen unsaturation of the venous blood. A previous investigation on the blood flow (minute volume) in patients with incompensated heart lesions (4) has shown a considerable decrease in the minute volume of the heart compared with that found in normal individuals

(5–14), and in most cases of compensated heart lesions[5] (4, 6, 11, 15). The figures obtained by Stewart (16) by determining the blood flow in the hands and feet point in the same direction. A retarded circulation, the rate of oxygen consumption not being decreased, may logically be assumed to result in a necessarily increased oxygen unsaturation of the average venous blood. The question is whether one is justified in assuming a retarded circulation from an increased oxygen unsaturation in the blood drawn from the vena mediana cubiti or from another superficial vein.

We know that other factors than the output from the heart may influence the extent of unsaturation in the blood from an arm vein. These factors are[6] (1) the possible unsaturation of the arterial blood; (2) variations in the metabolism of the tissues drained by the vein tapped as compared with the rest of the body; (3) variations in the rate of blood flow through the tissues drained compared with the rest of the body.

We are unfortunately, for the time being, only to a limited extent able to measure, control, or eliminate the influence of these factors.

(1) The dissociation curve for oxyhemoglobin shows that the blood must become nearly saturated (usually about 96 to 98 per cent) during the passage through the lungs, provided that all the blood flowing through comes into equilibrium with the alveolar air. This has been proved experimentally on animals by several investigators, and in

[5] Plesch (6) and Means and Newburgh (11) found normal figures for the blood flow in all their patients with compensated heart lesions, whereas Lundsgaard (4) found diminished minute volume not only in incompensated cases but in some patients with compensated heart conditions (mitral stenosis and auricular fibrillation), which agrees with Stewart's (16) observations for the local blood flow.

[6] One condition which might theoretically affect the oxygen saturation of the hemoglobin in arterial blood is incompensated acidosis, defined by Hasselbalch and Gammeltoft (17) and Van Slyke and Cullen (18), in which increased free carbonic acid causes an increase in the actual hydrogen ion concentration of the blood. As shown by Barcroft (19) this increase reduces the percentage oxygen saturation of hemoglobin under a given oxygen tension. Since, however, the differences thus caused in oxygen saturation become considerable only under reduced oxygen tension, it appears improbable that this factor, even in the infrequently occurring incompensated acidosis, exerts a significant effect on the degree of saturation of the arterial hemolgobin.

man it has been investigated by Hürter (20). By drawing blood from a radial artery and using Barcroft's method for determination of the oxygen, he found in four normal individuals a saturation of 94, 92, 99, and 100 per cent. In four patients with compensated heart lesions the saturation was 90, 100, 90, and 95 per cent. In one patient with incompensated aortic insufficiency and stasis bronchitis in the lungs the saturation was 81 per cent. In another patient with incompensation (Pick's disease) it was 92 per cent. A patient with patent ductus Botalli showed 88 per cent saturation. One with diffuse bronchitis showed 94 per cent, and another with phthisis, 88 per cent. Two patients with lobar pneumonia showed 81 and 79 per cent saturation. Barcroft[7] found a saturation of 94 per cent in a normal individual.

In several of my patients with compensated heart lesions moist râles have been heard at the base of the lungs. This circumstance does not seem to have influenced substantially the amount of oxygen in the venous blood. These râles are probably to a great extent just a proof that the air really does go down into the alveoli of the lowest part of the lungs. The whole problem is not sufficiently investigated to allow a decision concerning the influence of the involvement of the lungs in the individual case. We may confine ourselves to making a careful examination of the lungs in every case, hoping that special investigation of the unsaturation in patients with lung diseases or experimental studies will give us sufficient information.

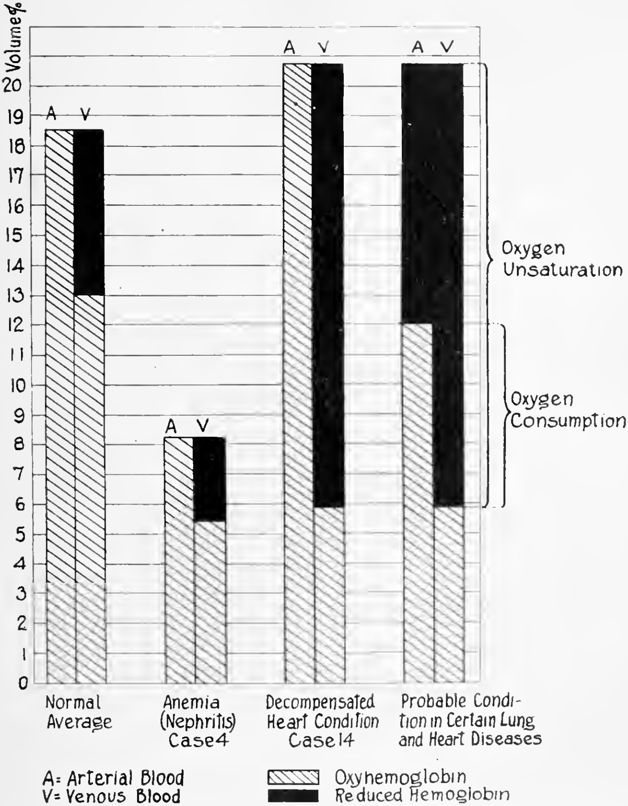
(2) The variations in the metabolism are presumably minimized by performing the experiments under definite conditions, such as muscular rest and digestive inactivity. The basal metabolism is a rather constant figure for the normal individual at rest and is, as shown by Lusk and his associates, chiefly dependent on the surface of the body. It has been shown by Peabody, Meyer, and Du Bois (21) that the metabolism in patients with compensated heart and cardiovascular lesions is within the normal limits. In patients with incompensated heart lesions, particularly in patients suffering from dyspnea, however, the metabolism was found materially increased, in some cases about 50 per cent. An increase in the metabolism necessarily means

[7] Barcroft (19), p. 177.

an increase in the consumption of oxygen. This, however, is not necessarily followed by an increase in the deoxidation of the oxyhemoglobin. That depends upon the output of the heart, which may or may not be changed. Lindhard (9), for instance, has shown that the increased consumption of oxygen during exercise only partially shows itself in the decreased oxygen in the venous blood; the increased blood flow compensates for a great part of the increased consumption. Lundsgaard (15) has shown that an increased oxygen consumption on account of exercise in two clinically compensated patients with heart block only to a very small degree could be compensated for by increasing blood flow. The reason for this was that it was impossible for the heart to increase the pulse rate and probably very difficult to increase the volume output per beat, which even in rest was about 150 cc. It is, therefore, probable that an increase in metabolism in a patient with a weak heart will increase the deoxidation of the venous blood, particularly in the venous blood coming from the heart muscle and the respiratory muscles. An increase in temperature may have a similar effect.

(3) About the only figures available on this point are those of Stewart (16). He has calculated from measurements of the heat given off by the hands and feet the rate of local blood flow and found that the flow in the right hand or foot is approximately equal to that in the left. Significant differences were encountered only when there was definite local cause, as aneurysms, diabetic gangrene, local edema, etc. It will probably be possible to throw light upon this question by simultaneously drawing blood from different veins and under different conditions, as tried in a number of cases by Means and Newburgh (11). Investigations on the local blood flow by Hewlett (22), using a modified Brodie method, have shown that there is a considerable temperature interval where the blood flow remains approximately constant when the surrounding temperature is changed.

The influence of the hemoglobin percentage, of the output from the heart, and of a possible unsaturation of the blood in the lungs is shown in diagram form in Text-fig. 2. The first double column represents the conditions in a person with normal hemoglobin, normal blood flow, and total saturation of the arterial blood. The next



TEXT-FIG. 2. Diagrams showing how the hemoglobin percentage, the output from the heart, and the saturation of the arterial blood influence the oxygen in the venous blood.

shows the findings in a patient with very low total oxygen capacity[8] but with normal circulation and full saturation in the lungs. A comparison of these two sets of figures shows the impossibility of using the zero as the base-line. The differences in hemoglobin must be accounted for. That is what we have done by using the term oxygen unsaturation, which is represented by the black area. The next two pairs of columns show how the extent of oxygen unsaturation can be affected in the same way by different causes. The third column represents the condition in a heart case (No. 14, Table II) when the total oxygen capacity was a little above normal; the saturation in the lungs was supposed to be normal. The cause of the increased oxygen unsaturation in this case was a slow circulation. The fourth column represents a hypothetical case with the same degree of oxygen unsaturation of the venous blood. However, the cause is ascribed to a considerable extent of unsaturation of the arterial blood. These two examples show the possible inaccuracy of using the word oxygen consumption in the same sense as oxygen unsaturation.

Oxygen Unsaturation Compared with Oxygen Consumption (Blood Flow).

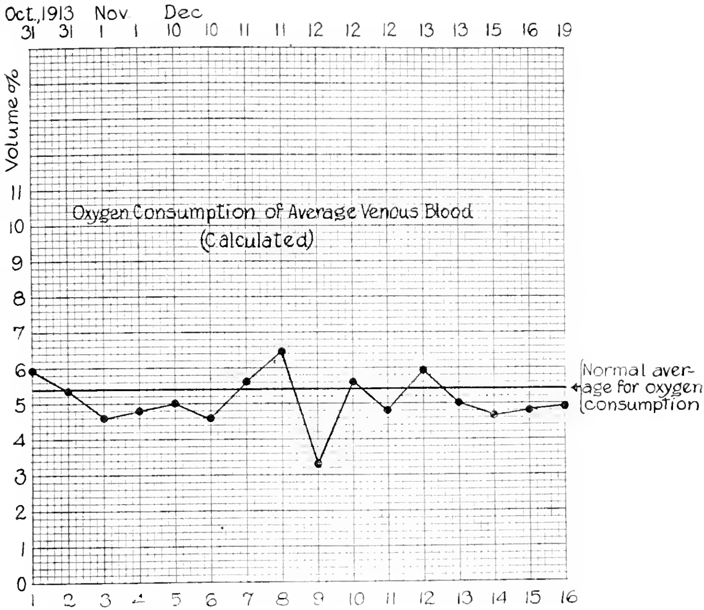
It is of interest for the interpretation of our results that the minute volume (output per minute from the heart) was determined 4 years ago in the Medical Clinic of the University of Copenhagen[9] on the same subject (the writer) on whom twenty determinations of the oxygen unsaturation of the venous blood have been done (Text-figs. 1 and 4).[10] From the figures indicating the values for the minute volume we can calculate the oxygen consumption of the average venous blood.[11]

[8] Compare Morawitz and Röhmer's observations (23).

[9] Lundsgaard (14), p. 397.

[10] See Table I, Paper I (1).

[11] The details of Krogh and Lindhard's method can be seen in the papers quoted in the bibliography, particularly in 8, 10, 13, and 14. The principles are the following: The subject is connected with an easily movable spirometer which contains a mixture of air and nitrous oxide. He mixes his lung air with the air in the spirometer during three to five respirations. He then stops breathing for a few seconds. Before and after the apneic period a sample of alveolar air is



TEXT-FIG. 3. Diagram showing the oxygen consumption of a normal man calculated from sixteen determinations of the minute volume of the heart by Krogh and Lindhard's nitrous oxide method.[12]

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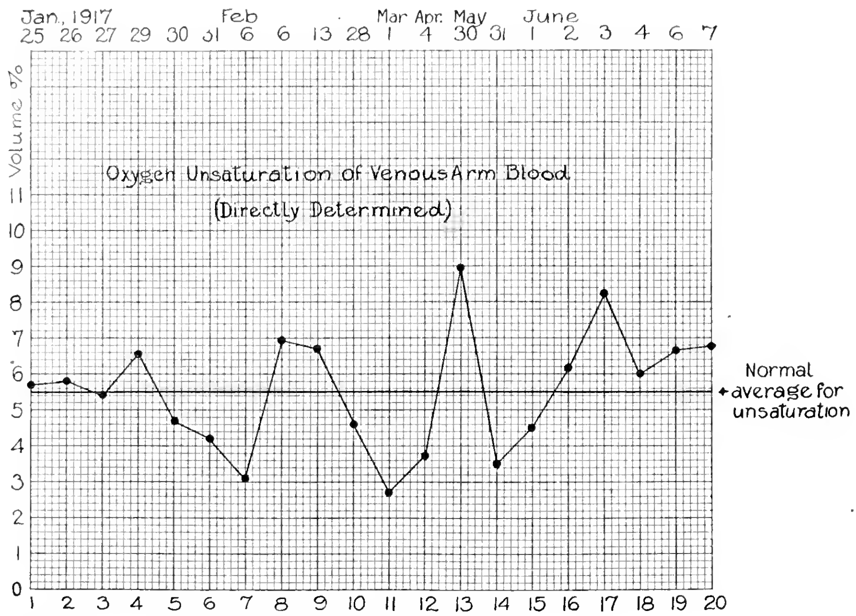
drawn and analyzed. On account of absorption by the blood flowing through the lung capillaries the second sample will contain a smaller amount of nitrous oxide and of oxygen than the first. The percentage in the difference between the two samples multiplied by the volume of air in the lungs will indicate the amount of blood flowing through the lungs during the period between the two samples. The output of blood per minute from the heart (the minute volume) can then be calculated. Knowing the quantity of oxygen taken up by the blood during the same period we can calculate the amount of oxygen taken up by the body per 100 cc. of blood. When a correction is introduced for a possible abnormality in the percentage of hemoglobin this amount will indicate the oxygen consumption by the average blood. An extensive critical study of Krogh and Lindhard's method is published by Sonne (24). He expresses doubt about the reliability of the method on account of possible imperfect mixture of the lung air, not only in patients but in normal individuals as well. Krogh and Lindhard (25) have later on admitted that the difficulties shown by Sonne may exist.

[12] See Lundsgaard (14), p. 397, where the data for the blood flow can be obtained.



TEXT-FIG. 4. Diagram showing the oxygen unsaturation of the venous arm blood from the same normal subject[4] as in Text-fig. 3 (see also Text-fig. 1).

Sixteen determinations of the blood flow were made at that time. The calculated figures for the oxygen consumption in these sixteen experiments are given in Text-fig. 3. The average oxygen consumption for a considerable number of blood flow experiments on normal people (5.4 volume per cent) is indicated.

In Text-fig. 4 are given the figures for the oxygen unsaturation of the venous arm blood of the same subject (the writer) as reported in the first paper of this series.[10] The average line in this diagram (5.5 volume per cent) is from thirty-eight determinations of the oxygen unsaturation on eleven normal people reported in Paper I and shown in Text-fig. 1 in this paper. It will be seen how closely the values for the oxygen unsaturation determined by Van Slyke's method agree with the values for the oxygen consumption calculated from the determinations of the blood flow with Krogh and Lindhard's method. The average figures for normal individuals are the same (5.5 and 5.4 volume per cent). The variations for the two series on the same person agree rather closely. The variations in the oxygen unsaturation are more extensive than the variations in the values for the average consumption; *i.e.*, for the blood flow. This is probably due to variations in the local blood flow in the arm from which the blood is drawn. It is worth mentioning that the subject on whom the determinations were done has a very labile circulatory system (respiratory arrhythmia, changeable pulse, dermographia).[13] The significance of this agreement is that the amount of oxygen lost by 100 cc. of blood in passing through the forearm is approximately the same as the average loss in passing through the other body tissues. In view, nevertheless, of the undoubted possibility that the disturbing factors discussed may influence the unsaturation, we are not justified at present in interpreting unsaturation figures in terms of minute volume of the heart. What we believe we can do, is to fix the limits of the oxygen unsatura-

[13] The conception that the extensive variations in the oxygen unsaturation in this particular case are principally due to vasomotor changes, is supported by an observation (Cohn and Lundsgaard, personal communication) on the relation between the brachial blood pressure and the blood pressure in the arteries of the finger. The tension in the digital arteries was found more variable than in other normal subjects and more variable than the blood pressure nearer the center.

tion in subjects with normal circulation and normal lungs and study the variations observed in carefully controlled patients with symptoms of abnormal circulation and in patients with respiratory abnormalities. From the data thus obtained we may empirically standardize the figures for the oxygen unsaturation, learn the pathological conditions that affect it, and thus add it to the armamentarium that assists the clinician in accurate diagnosis. The empirical evolution of blood pressure measurement, made possible by accumulation of many determinations on clinically controlled patients, has shown how a quantitatively measurable factor, even though imperfectly explained physiologically, may prove to be of value in clinical medicine.

SUMMARY.

1. Thirty-one determinations of the total oxygen-combining power and the oxygen in the venous blood from vena mediana cubiti of sixteen resting patients are reported.

2. The difference between the total oxygen capacity of the hemoglobin and the oxygen in the venous blood, the oxygen unsaturation, is calculated.

3. In twelve patients with compensated heart lesions the unsaturation was found within normal limits, between 2.5 and 8 volume per cent.

4. In four patients with incompensated heart disease the values for the unsaturation were all above the normal limit, from 9.7 to 15.2 volume per cent.

5. A general discussion of the problem of interpreting the results is given.

6. A comparison is drawn between the oxygen consumption calculated from direct determination of the blood flow on a normal subject (the writer) and the oxygen unsaturation determined 4 years later on the same subject. A close agreement between the two series of values exists.

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STUDIES OF OXYGEN IN THE VENOUS BLOOD.

III. Determinations on Five Patients with Compensated Circulatory Disturbances.

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INTRODUCTION.

In two previous papers on the same subject (1, 2) the technique of drawing blood from the arm vein and a series of determinations on twelve normal individuals and sixteen patients with circulatory disturbances have been reported.

In the discussion of the interpretation of the results, it was pointed out that variations in the oxygen unsaturation of the blood from an arm vein might be caused by several factors other than the output from the heart, which we were able to control or eliminate only to a limited degree. These factors were: (1) the degree of oxygen saturation of the arterial blood; (2) the oxygen consumed by the metabolism of the part of the body drained by the vein tapped; (3) changes in the local blood flow on account of vasodilatations or constrictions either in the organ (the arm) from which the blood is drawn or in other parts.

In animal experiments the influence of these factors might be studied separately. In clinical medicine, however, we have to deal with a given condition where several factors usually act together. We are, therefore, compelled to compare our findings with the clinical observations and postpone the conclusions until sufficiently extensive material has been collected.

The purpose of these investigations is to study the variations in the oxygen unsaturation of the venous blood in patients with symptoms of circulatory disturbances; that is, in patients in whom it is of importance to find out whether the blood flow is changed or not.

The preliminary determinations reported in Paper II gave promising results. Continuous determinations on patients over a longer period have therefore been taken up. Ten patients have been studied, five with compensated[1] and five with incompensated circulatory disturbances. This paper is a report of the findings in the compensated group.

Before reporting the clinical notes and the figures for the oxygen unsaturation in these five patients, one should recall that the limits for the oxygen unsaturation in normal individuals were 9 and 2.5 volume per cent. However, values between 8 and 9 volume per cent were found in only four determinations and under special circumstances—a few seconds after awakening from a night's sleep, a condition which, as a rule, we shall be unable to reproduce in patients. Considering that all the values for the oxygen unsaturation in twelve patients with compensated circulatory disturbances (2) fell between 8 and 2.5 volume per cent, it seems correct, at least for the time being, to consider 8 volume per cent the upper normal limit. The lower limit is 2.5 volume per cent, the average 5.5. In the diagrams of the oxygen unsaturation lines have been drawn to indicate the normal limits.

CASES.

Case 1 (Table I, Text-Fig. 1).—V. G., male, gardner; age 58 years.

Diagnosis.—Aortic insufficiency; arteriosclerosis; hypertension.

Previous History, Symptoms, etc.—Dyspnea after slight exertion, for 1 year. He has never had syphilis or rheumatic fever, but for years he has suffered from stiffness of the back and has now and then had slight joint pains.

Physical Examination.—No cyanosis; no swelling of superficial glands; lungs clear; heart dullness increased considerably to the left and downwards; x-ray shows increase to the left and downwards; no thrill. At the fourth rib on the left side is heard a distinct diastolic murmur replacing the second sound, also heard at apex; liver not felt; no ascites; no edema. In the urine was found a trace of albumin; no casts. Blood pressure 150–170. Wassermann reaction negative. Temperature normal.

He was admitted to the hospital for diagnosis, confined to bed for the first 3 days, and after that time allowed to get out of bed. No special treatment. He felt better when discharged; dyspnea less; physical signs unchanged.

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[1] Patient 4 in the present paper had symptoms of heart failure. During the course of the disease the symptoms were interpreted as not circulatory.

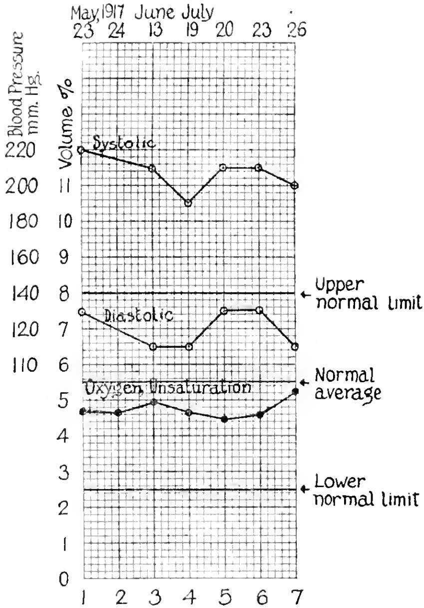
TABLE I.

Case. 1. Aortic Insufficiency; Arteriosclerosis; Hypertension; Hypertrophy of Left Side of Heart.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a − v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.*	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	May 23	1.00 p.m.	Left.	¾ hr.	2.30	13.90	2.50	13.82	13.86	100	18.50	4.64	72	24
2	" 24	1.00 "	Right.	¾ "	3.00	14.29	3.10	13.64	13.97		(18.50)	4.53	76	20
3	June 13	11.50 a.m.	"	¾ "	12.20	13.55	12.50	13.46	13.51	100	18.50	4.99	66	24
4	July 19	11.00 "	Left.	1½ hrs.	11.20	14.01	12.00	14.89	14.45	103	19.06	4.61		22
5	" 20	11.50 "	"	18 "	1.10	14.68	1.30	14.68	14.68	103	19.06	4.48	66	22
6	" 23	9.30 "	Left and right.	12 "	10.20	14.60	10.50	14.32	14.46	103	19.06	4.60	66	22
7	" 26	9.40 "	Right.	½ hr.	10.20	13.82	10.40	13.62	13.72	103	19.06	5.34	72	24

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

This patient, who suffered from a valvular heart lesion which is fully compensated at rest, shows almost constant values for the oxygen unsaturation from day to day. Furthermore the values follow the normal average. He was a patient with a quiet temper, with very slowly reacting vasomotors and with a steady, regular pulse.



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of aortic insufficiency; arteriosclerosis; hypertension.

Case 2 (Table II, Text-Fig. 2).—T., male, steward; age 52 years.

Diagnosis.—Hypertension arterialis; slight arteriosclerosis; chronic alcoholism; cirrhosis of the liver (?); lues.

Previous History, Symptoms, etc.—Shortness of breath on exertion, for 4 years.

Physical Examination.—Rather fat man; lungs clear; no cyanosis; no prominent

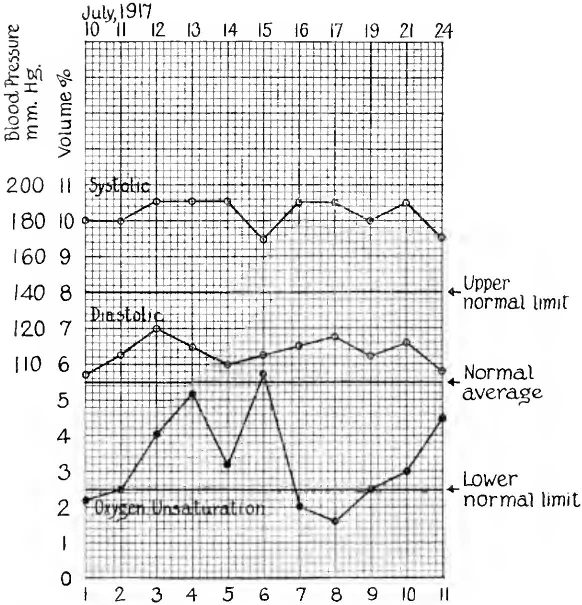
TABLE II.

Case 2. Arterial Hypertensions; Slight Arteriosclerosis; Cirrhosis of Liver; Lues; Chronic Alcoholism.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Pulse.	Respirations.
	Date	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	July 10	9.30 a.m.	Left.	In bed.	10.30	17 02	10.50	16 80	16 91	103	19 06	2 16	73	20
2	" 11	9.30 "	"	" "	4.10	16 62	4 40	16.62	16 62	103	19 06	2 44	84	24
3	" 12	9.30 "	Right.	" "	10.10	15 05	10.50	15.05	15 05	103	19 06	4 01	87	20
4	" 13	9.30 "	"	" "	11 10	13.83	3.40	13.99	13.91	103	19 06	5 15	92	28
5	" 14	9.30 "	"	12 hrs.	2.10	14 60	2.20	14.80	14.70	97*	17.92	3 22	92	22
6	" 15	12.20 p.m.	"	12 "	12 40	12 07	12.55	12.81	12.44	98*	18 13	5 69	91	22
7	" 16	10.30 a.m.	"	12 "	10.40	16.85	10.53	16.85	16.85	102	18 82	1 97	90	24
8	" 17	9.20 "	"	12 "	10 20	17.50	11.00	17.40	17.45	103	19 06	1.51	92	28
9	" 19	9.30 "	Left.	12 "	10 00	15.97	10.20	15 97	15.97	100	18 50	2.53	98	22
10	" 21	9.30 "	"	12 "	9 40	15.82	10.10	15.18	15.50	100	18 50	3 00	96	24
11	" 24	9.30 "	Right.	1 hr.	10 00	13 25	10 15	13 25	13.25	96	17.74	4 49		26

* Checked three times.

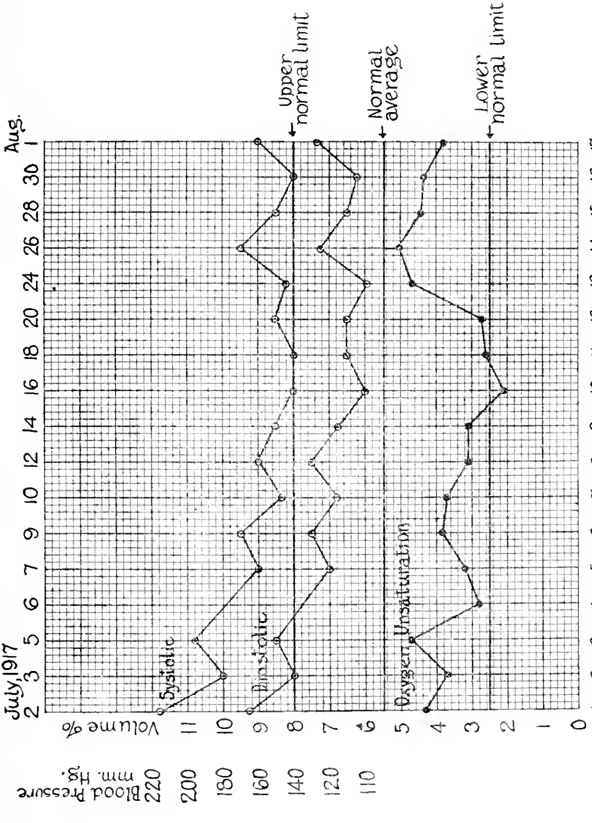
veins; heart covered by the lungs; limits uncertain at percussion; x-ray shows the heart moderately enlarged to the left; no murmurs; aortic second sound high pitched and accentuated. The abdomen is fat with prominent veins on both sides from the inguinal region to the axilla. Liver not felt; area of dullness over spleen not distinctly increased; no jaundice; no ascites; no edema; the urine con-



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of arterial hypertension; slight arteriosclerosis; cirrhosis of liver; lues.

tains a faint trace of albumin; no casts; blood urea not increased. Wassermann reaction positive. Temperature normal.

Treatment.—Confined to bed; restricted fluid and calories; no medication apart from antisyphilitic treatment, which was started after the determinations of the oxygen were finished.



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of arteriosclerosis; hypertension; lues; aneurysm; aortic insufficiency.

TABLE III.

Case 3. Arteriosclerosis; Hypertension; Lues; Incipient Aneurysm of the Aorta.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a - v).	Pulse.	Respirations.
	Date	Hour	Arm	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour	Result.*	Hour.	Result						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	July 2	2.00 p.m.	Left.	1 hr.	2.10	17.14	2.30	17.32	17.23	116	21.48	4.25	108	20
2	" 3	9.30 a.m.	"	In bed.	10.00	17.63	10.30	17.86	17.75	116	21.48	3.73	80	14
3	" 5	11.30 "	"	" "	12.00	16.46	1.00	17.02	16.74	116	21.48	4.74	88	20
4	" 6	9.50 "	"	" "	4.40	18.64	5.20	18.64	18.64	116	21.48	2.84	88	20
5	" 7	9.20 "	"	" "	9.40	18.24	10.20	18.44	18.34	116	21.48	3.22	94	24
6	" 9	11.00 "	"	" "	11.50	18.44	12.00	18.22	18.33	120	22.20	3.87	80	20
7	" 10	4.30 p.m.	"	" "	5.30	15.56	6.00	18.54	18.55	120	22.20	3.65	80	22
8	" 12	9.20 a.m.	"	" "	11.00	18.95	11.20	20.05	19.50	122	22.54	3.04	80	22
9	" 14	9.40 "	"	½ hr.	11.00	17.45			17.45	111*	20.54	3.09	81	20
10	" 16	9.30 "	"	½ "	10.00	18.44	10.30	18.50	18.47	111	20.54	2.07	86	24
11	" 18	9.50 "	"	½ "	11.30	16.86	11.50	17.30	17.08	106	19.62	2.54	84	24
12	" 20	9.30 "	Right.	½ "	9.50	17.03	10.20	17.24	17.14	107	19.80	2.66		
13	" 24	9.30 "	Left.	½ "	11.00	14.53	11.30	14.53	14.53	104	19.25	4.72	88	26
14	" 26	9.30 "	"	½ "	10.50	14.22			14.22	104	19.25	5.03	96	20
15	" 28	9.30 "	Right.	½ "	9.50	14.84			14.84	104	19.25	4.41	72	20
16	" 30	9.30 "	Left.	½ "	9.50	13.92	10.30	14.34	14.13	100	18.50	4.37	80	24
17	Aug. 1	9.30 "	Right.	½ "	9.50	14.88	10.30	14.54	14.71	100	18.50	3.79	94	24

* The determination of the hemoglobin was repeated three times on the blood in which the oxygen was determined. Furthermore, a sample was drawn from the ear, which gave the same result. The standard was also tested.

This patient was a moderately advanced case of the cardiovascular type. Besides he probably had some cirrhosis of the liver. His circulation was fully compensated. The oxygen unsaturation shows less constancy than in Case 1. However, the variations extend practically only over the half of the area which is covered by the values for the normal individuals. The variations are smaller than in the normal person and the curve as a whole is more uniform.

Case 3 (*Table III*, *Text-Fig. 3*).—L., male, musician; age 35 years.

Diagnosis.—Arteriosclerosis; hypertension; lues; incipient aneurysm of the aorta.

Previous History, Symptoms, etc.—Headache; shortness of breath on exertion; contracted syphilis 6 years ago; treated regularly.

Physical Examination.—The site of the heart is normal to percussion and x-ray; no murmurs; both aortic and pulmonic second sounds very distinct; aortic particularly high pitched; heart action regular; electrocardiogram normal. The x-ray picture shows a slight distention of the left side of the upper part of the descending aorta. Wassermann test doubtful three times. Lungs clear; no cyanosis, distended veins, or edema; liver not felt; radial and temporal arteries tortuous and very hard; urine negative for albumin. For the blood pressure see Text-fig. 3. Temperature normal.

Treatment.—Confined to bed for the first 12 days; salt-free diet; restricted calories and fluid.

During his stay in bed his hemoglobin increased from 116 to 122. The 2nd day he was out of bed 3 hours, his hemoglobin dropped suddenly to 111 (verified several times on two different samples of blood) and then slowly down to 100 per cent. No explanation for this could be found in his condition or treatment.

We have here a patient with a pronounced premature arteriosclerosis, probably on syphilitic basis. Besides that he had a beginning aneurysm which could be detected only by x-ray. His shortness of breath on exertion is probably due entirely to this high blood pressure. The values for the oxygen unsaturation in seventeen determinations are rather constant and, like the values in Case 2, occupy the space below the normal average, one a trifle below the normal lower limit.

Case 4 (*Table IV*, *Text-Fig. 4*).—J. A., male, porter; age 49 years.

Diagnosis.—Cirrhosis of the liver; nephritis; urea retention; uremia (?); anemia.

Previous History, Symptoms, etc.—Edema of legs for 3 to 4 months; has used much alcohol; has never had any sickness before.

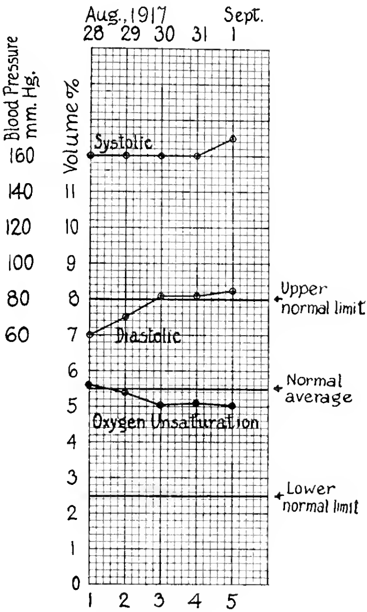
Physical Examination.—Dullness from the right border of sternum to 2 cm. below the nipple. X-ray shows that the heart is in a transverse position (on account of his ascites); the size and form not changed. All over the precordia a

TABLE IV.

Case 4. Nephritis; Uremia; Cirrhosis of Liver; Anemia.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a - v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 28	9.30 a.m.	Right.	In bed.	9.50	7.80			7.80	72	13.32	5.52	79	22
2	" 29	9.30 "	"	" "	9.50	7.90	10.10	7.90	7.90	72	13.32	5.42	78	22
3	" 30	4.00 p.m.	"	" "	4.10	8.16	4.30	8.36	8.26	(72)	(13.32)	5.06	94	26
4	" 31	10.00 a.m.	"	" "	10.20	7.85	10.40	7.85	7.85	70	12.94	5.09	78	28
5	Sept. 1	6.50 "	"	" "	8.20	8.28	8.40	8.28	8.28	72	13.32	5.04	96	24

soft systolic murmur;[2] aortic and pulmonic second sounds normal; pulse regular; electrocardiogram normal; the day of the first determination some moist râles in lungs; since then clear; no cyanosis; veins are not distended; marked ascites; no jaundice; liver and spleen not felt, but the area of dullness over the spleen is distinctly increased; uremic odor of breath; sleepy; heavy edema of lower extremi-



TEXT-FIG. 4. Oxygen unsaturation of venous arm blood. Case of cirrhosis of liver; nephritis; uremia; anemia.

ties extending up over the lower part of abdomen; diuresis small. Urine contains albumin; granular and hyaline casts; urea in blood somewhat increased (50 mg. of urea per 100 cc. of blood). Condition stationary during the time of bleeding.

Treatment.—Confined to bed; 800 cc. of milk per day. Diuresis very small (200 to 400 cc.). For the blood pressure see Text-fig. 4.

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[2] He was admitted as a case of incompensated heart failure.

The diagnosis in this case was confirmed by an autopsy which showed a typical liver cirrhosis of Laennec's type, a big hard spleen, parenchymatous, subchronic nephritis; heart and lungs were normal; no sclerosis of aorta. The results on this patient show the importance of using the oxygen unsaturation, not the direct value for the oxygen in the venous blood, on account of the anemic condition. A consideration of the whole clinical picture made it obvious that his ascites, edema, and low diuresis were not due to incompensated circulation. It is, therefore, interesting, to find normal values for the oxygen unsaturation. The values are not only within the normal limits, but they are very constant and follow absolutely the line for the normal average. The day when the first determination was made quite a number of moist râles were heard at the base of the left lung. At the time of the following determinations the lungs were clear. However, no differences were observed in the figures for the oxygen unsaturation.[3] He was very quiet all the time, in fact his mind was not quite clear, particularly at the time of the last determinations. The temperature was normal. He died 5 days after the last determination.

Case 5 (Table V, Text-Fig. 5).—G., male, teacher; age 38 years.

Diagnosis.—Mitral insufficiency; cardiac palpitations; auricular fibrillation.

Previous History, Symptoms, etc.—Palpitation; fatigue on exertion, for 4 to 6 years; no rheumatic fever; no syphilis.

Physical Examination.—On admission moist râles at the base of the left lung; otherwise lungs are clear; no cyanosis or swelling of superficial vein; dyspnea only on rather considerable exertion; dullness distinctly increased to the left and in direction of the left axilla; at apex distinct systolic murmur replacing almost entirely the first sound; no diastolic murmur; pulse rate moderately rapid (100); electrocardiogram and pulse show auricular fibrillation; liver not felt, not tender; no edema. Temperature normal. From April 19 to 30 he had 0.5 gm. of digipuratum a day. He did not show any clinical signs of incompensations. The râles in his lungs disappeared and the lungs were clear at the time of the determinations; temperature normal.

The results show two very important traits. In the first place, the values for the oxygen unsaturation vary to an extent not seen before, from 4.5 to 16.2 volume per cent. In the second place, he is the first patient with a clinically compensated heart lesion in which there have been found values for the oxygen unsaturation outside the normal limits, 8 and 2.5 volume per cent. The results on this patient are in striking contrast to those found in the other four patients.

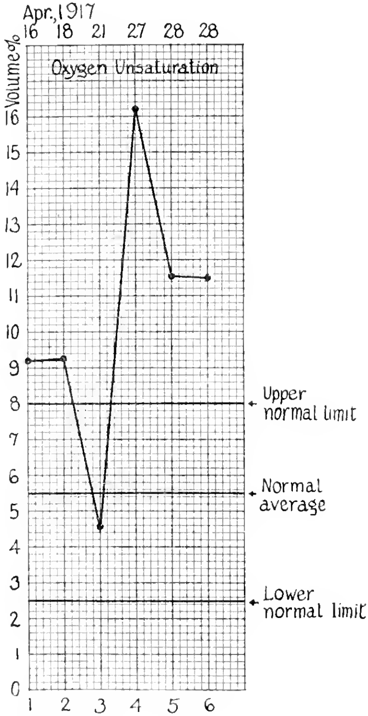
[3] See the discussion in Paper II (2) about the saturation of the arterial blood.

TABLE V.

Case 5. Mitral Insufficiency; Cardiac Palpitations; Auricular Fibrillation.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a − v).	Pulse.		Respirations.
	Date.	Hour.	Arm	Condition.	Sample 1.		Sample 2.		Average (v).				Apex.	Radial.	
					Hour.	Result.	Hour.	Result.							
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*			
1	Apr. 16	3.00 p.m.	Right.	In bed.	3.40	10.00	3.55	10.08	10.04	104	19.24	9.20		84	20
2	" 18	11.30 a.m.	"	" "	12.10	9.91	12.30	10.25	10.08	104	19.30	9.22		64	20
3	" 21	11.40 "	"	2 hrs.	2.40	15.71	2.55	15.75	15.73	118	21.28	4.55	87	72	19
4	" 27	3.10 p.m.	"	1 hr.	3.30	5.81	3.50	6.25	6.03	120	22.20	16.17	74	64	20
5	" 28	11.00 a.m.	Left.	3 hrs.	12.00	9.01	12.30	9.27	9.14	114	20.70	11.56	86	84	22
6	" 28	11.15 "	"	3 "	12.15	9.27	1.30	9.11	9.18	(114)	(20.70)	11.52	86	81	21

The most conspicuous clinical difference between him and the other four patients is the heart rhythm. It is difficult to find other cause for the change in the oxygen unsaturation than variations in the output from the heart. The lungs were clear, there was no evidence of any



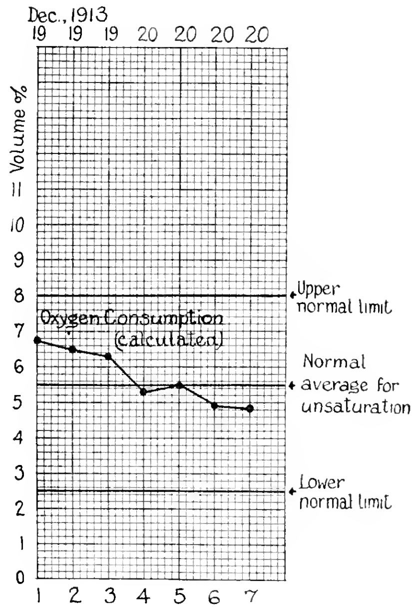
TEXT-FIG. 5. Oxygen unsaturation of venous arm blood. Case of mitral insufficiency and auricular fibrillation.

changes in the metabolism, and it seems unlikely that undetected vasomotor changes could account for the fluctuations shown in the chart.

DISCUSSION.

A previous investigation of the minute volume in heart patients by the writer (3) gave results which agree closely with those found here.

It was found that patients with valvular disease combined with arrhythmia perpetua or with groups of extrasystoles may show (1) abnormally great variations in the output and (2) diminished minute volume without clinical signs of incompensation. It is possible to calculate the degree of oxygen consumption[4] in the patients referred



TEXT-FIG. 6. Case of compensated mitral and aortic insufficiency and mitral stenosis. Pulse regular.[5] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

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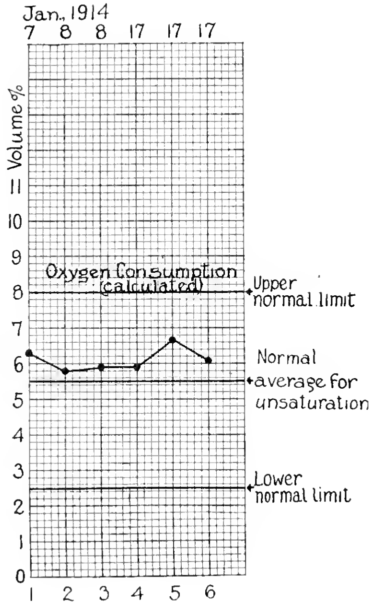
[4] The difference between oxygen unsaturation and oxygen consumption is defined in Papers I and II (1, 2). In Paper II is given a short description of Krogh and Lindhard's method.

[5] Case 1, Lundsgaard (3), p. 518.

to. Text-figs. 6 to 9 show the calculated values for the oxygen consumption in four patients where the minute volume was determined by Krogh and Lindhard's nitrous oxide method. Text-figs. 6, 7, and 8 represent patients suffering from compensated heart lesions. The

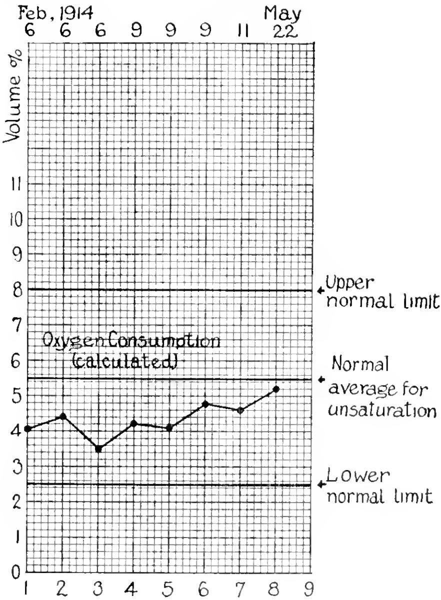


Text-Fig. 7. Case of compensated mitral stenosis and insufficiency. Pulse regular.[6] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

pulse rate was regular in these cases. It will be seen that the figures agree closely with the directly determined oxygen unsaturation in the first four patients in this paper.

[6] Case 10, Lundsgaard (3), p. 544.

Text-fig. 9, on the other hand, represents a patient with clinically compensated mitral stenosis and auricular fibrillation. The calculated values for the oxygen consumption (1) are varying, (2) are above the normal line, and (3) agree closely with the directly determined

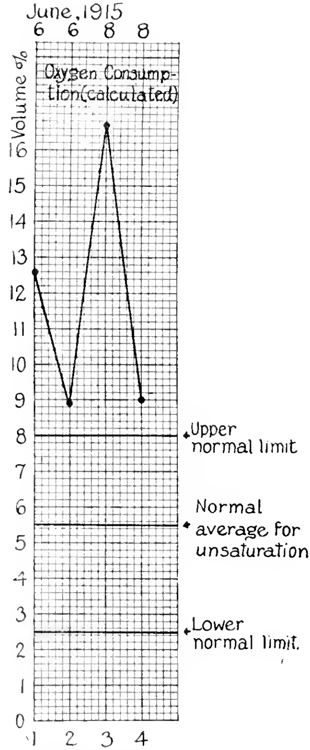


TEXT-FIG. 8. Case of compensated heart block.[7] Pulse regular. Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

values in Case 5 of this report. This seems to be sufficient proof that the variations in the oxygen saturation of the venous arm blood may in some instances express the variations in the output of the heart.

[7] Case 1, Lundsgaard (4), p. 488.

The values for the oxygen unsaturation in the first four patients are, as mentioned before, more uniform than the values in normal



TEXT-FIG. 9. Case of compensated mitral stenosis. Pulse irregular. Auricular fibrillation.[5] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

[5] Case 7, Lundsgaard (3), p. 535.

people. The same thing could be seen in the determinations of the minute volume of the heart in normal people and in patients with compensated circulatory disturbances. The figures for the oxygen unsaturation calculated from the blood flow were more regular in patients with compensated valvular disease and regular heart action than in the two normal people. This may be interpreted as indicating that a regularly beating heart working against a certain burden will have a smaller margin of action than the normal heart. The burden probably acts on the heart like a heavy wheel on a machine, making its action steady.[9]

SUMMARY.

1. Forty-six determinations of the oxygen unsaturation, *i.e.*, the difference between the venous oxygen and the total oxygen capacity of the hemoglobin, have been done in five patients with compensated circulatory disturbances.

2. Values within normal limits and near or below the normal average were found in four patients with regular pulse.

3. In one patient with mitral insufficiency and arrhythmia perpetua extremely varying values were encountered. Six determinations were made; in one instance the value was within normal limits, in five above, and in one of those even higher than in most cases of incompensated heart lesions.

4. A comparison is drawn between the directly determined oxygen unsaturation in these patients and the oxygen consumption calculated from previous experiments by the writer, where the blood flow was determined directly by the nitrous oxide method. A close parallelism is found.

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[9] It is worth mentioning that the normal person on whom twenty determinations of oxygen were done (Paper I and Text-fig. 1, Paper II) had a very unsteady circulation (unsteady pulse, respiratory arrhythmia, dermographism). Great variations in the oxygen unsaturation were encountered.

STUDIES OF OXYGEN IN THE VENOUS BLOOD.

IV. Determinations on Five Patients with Incompensated Circulatory Disturbances.

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INTRODUCTION.

The technique of drawing and preserving the blood, the results on normal individuals, and a preliminary study of patients with circulatory disturbances have been reported in Papers I and II (1, 2) of this series.

In accordance with the viewpoints laid down in these two papers an investigation of the variations in the oxygen unsaturation[1] of the venous blood over a longer period in the same person was taken up. Ten patients suffering from different forms of circulatory disturbances have been studied. In Paper III (3) a report was made of 46 determinations on five patients in a compensated stage. The present paper is a report of 103 determinations on five patients with different heart lesions, all of whom, during a certain period, were distinctly clinically incompensated. Repeated determinations have been made during the course of the disease, and the clinical symptoms which have bearing upon the compensation or incompensation have been noted.

Short descriptions of the history, the physical examination at admission, and the treatment are given. The development of the disease can be followed from the notes on the charts. In the diagrams are given the curves of the oxygen unsaturation of the venous blood.

[1] The term oxygen unsaturation means the difference between the total oxygen-combining power of the hemoglobin and the oxygen in the venous blood. For further details see the discussion in the preceding papers, especially Paper II, Text-fig. 2.

The extreme normal limits and average normal are indicated on the diagrams for comparison. Marks showing the treatment with digitalis and the condition of the circulation (compensation or incompensation) are added. The clinical symptoms, which have been especially followed, are dyspnea, cyanosis, swelling of the superficial veins, enlargement of the liver, edema, diuresis, and body weight. The figures for the respiration indicate the rate just after the blood has been drawn. For that reason the rate of respiration is sometimes somewhat higher than would be the case if the patient had not been bled. This holds particularly true in the compensated stage.

The pulse has been counted during the bleeding. In patients with auricular fibrillation the apex rate and radial rate have been counted simultaneously in order to obtain the pulse deficit. In the few instances where the temperature of the patients has been increased, it is noted in the description of the patient.

CASES.

Case 1 (Table I, Text-Fig. 1).—P., male, photographer; age 46 years. Admitted March 26, 1917. Discharged April 30.

Diagnosis.—Myocarditis; arteriosclerosis; chronic nephritis; hypertension.

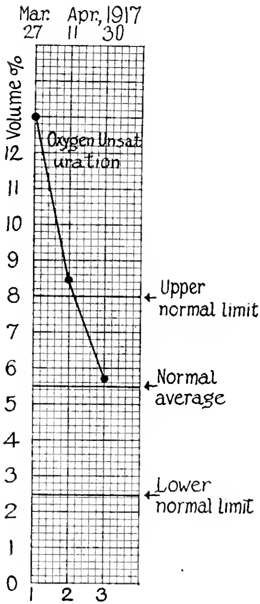
Previous History, Symptoms, etc.—Shortness of breath for a year, particularly on exertion; swelling of legs and abdomen for 2 months. No syphilis or rheumatic fever. He has used alcohol every day for several years.

Physical Examination.—Heart dullness and shadow on x-ray picture increased to the left and downwards. Limit of dullness is 3.5 cm. to the right and 14 cm. to the left of the middle line at the fourth interspace, 15 cm. to the left at the sixth interspace. An indistinct systolic murmur is heard all over the precordium. Aortic second sound is accentuated. Blood pressure: systolic 175, diastolic 125. There are moist râles and slight dullness at the lower parts of both lungs. He is cyanotic and the veins of the neck are distended. There is distinct ascites; the liver is felt, not tender. Marked edema of both legs extending up over sacrum. In the urine was found slight albumin, and occasionally granular casts. Blood urea considerably increased (73 mg. per 100 cc.). Index of urea excretion (McLean (4)) 15.0, in the course of the treatment increasing to 74.0. Wassermann test negative.

Treatment.—He was confined to bed and treated first with milk diet, and later with salt-free diet.

Three determinations of the oxygen unsaturation were done. The first was done the day after admission. He was distinctly incompensated and the value for the unsaturation was far above the upper normal

limit. The second determination was done at a time when it was doubtful whether he was compensated or not (Table I and Text-fig. 1). The oxygen unsaturation was at that time just above the upper normal limit. At the time of the third determination he was absolutely compensated



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of myocarditis; arteriosclerosis; chronic nephritis; hypertension.

at rest.[2] His oxygen unsaturation was at that time normal. The interpretation of this case is made difficult by the fact that there were quite a number of râles in the lungs at the time of the first determination, which may or may not have affected the saturation of the

[2] In the evening a slight edema around the ankles was found.

TABLE I.

Case 1. Myocarditis; Arteriosclerosis; Chronic Nephritis; Hypertension.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.‡									
	Date.	Condition.*	Sample 1.	Sample 2.†	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Mar. 27	In bed.	7.29	7.74	7.52	111	20.52	13	88	26§	++	++	++	+	+++	950	310	59.0
2	Apr. 11	½ hr.	14.69	14.67	14.68	125	23.15	8.47	89	17‖	−	−	−	−	+	1,500	575	47.6
3	" 30	½ "	12.48	13.00	12.74	100	18.50	5.76	98	22¶	−	−	−	−	−	1,500	625	51.8

TABLE II.

Case 2. Mitral Stenosis and Insufficiency; Mitral Stenosis (?); Recurrent Endocarditis (?).

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Aug. 30	In bed.	4.50	5.00	4.75	71	13.13	8.38	108	34**	++	++	+++	+++	+	1,000	484	38.4
2	Sept. 4	" "	4.60	4.80	4.70	70	12.94	8.24	104	32	++	++	+++	+++	+	1,000	420	38.7
3	" 7	" "	2.30	2.20	2.25	67	12.38	10.13	100	28	++	++	+++	+++	?	1,000	740	39.7
4	" 10	" "	4.94	5.12	5.03	71	13.13	8.10	94	32	++	++	+++	+++	?	1,000	676	40.2
5	" 12	" "	3.62	3.80	3.71	71	13.13	9.42	90	24	++	++	+++	+++	+	1,000	565	39.8
6	" 14	" "	4.81	4.81	4.81	71	13.13	8.32	104	32	++	++	+++	+++	+	1,000	670	40.4

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

† The interval between the two samples given in all the tables was generally about 15 to 20 minutes.

‡ The severity of these symptoms is indicated by means of crosses. Three stages are distinguished. Three crosses indicate a dyspnea (orthopnea) during which the patient was sitting almost straight up in bed in order to breathe. One cross means a just appreciable dyspnea, and two crosses mean an intermediate stage.

One cross for cyanosis means that only the lips were bluish, two crosses indicate that the ears and skin of the face were cyanotic, and three crosses mean a rather dark blue color of lips, face, and skin of the hands.

A just appreciable swelling of the veins in the neck and the dorsum of the hands is indicated by one cross. Distinct and prominent swelling of the veins of the neck, arms, and hands is indicated by three crosses. Two crosses represent an arbitrary medium stage.

As far as the liver is concerned, one cross means that it can be distinctly felt below the curvature; two crosses indicate that it extends 3 to 6 cm. downwards; and three crosses indicate that it extends still further, and is usually rather tender.

One cross for the edema indicates a little swelling on the dorsum of the foot around the ankles, or over the inside of the tibia. Two crosses indicate a distinct swelling of feet and legs, and three crosses indicate that the edema has extended up over the femurs and hips.

It will be understood that it has been difficult to obtain a satisfactory classification of these symptoms. A question mark indicates that in some instances it has been impossible to decide whether a symptom has been absent or present.

§ Some moist râles and slight dullness at the base of the lungs.

‖ The lungs were clear except for a few crackling râles at the left axilla.

¶ Lungs clear.

** Lungs clear at each determination.

arterial blood.[3] In several instances (Papers II and III) the oxygen unsaturation has been normal in patients, where just as many râles were heard as in this case. At the time of the second determination a few crackling râles in the left axilla were noticed. These disappeared the next day.

The edema in this case was supposed to be chiefly of nephritic origin. Besides this it was evident that he had an incompensated myocarditis. The results of the oxygen determinations in this case at the different stages of the disease agree therefore with what was previously found in patients with compensated and incompensated heart lesions (Papers II and III).

Case 2 (Table II, Text-Fig. 2).—M., male, student; age 14 years. Admitted August 28, 1917. Discharged October 21.

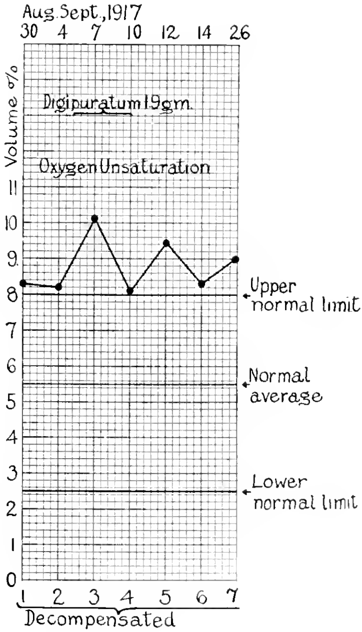
Diagnosis.—Mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis (?).

Previous History, Symptoms, etc.—Palpitation and dyspnea, particularly on exertion, for 3 years. He has had four attacks of rheumatic fever, the first 6 years ago. The second, 3 years ago, involved his heart. He had, furthermore, an attack of chorea 2 years ago. During the last 2 months, since the last hospital treatment for rheumatic fever and endocarditis, he has suffered from increasing palpitation and dyspnea. He has been confined to bed during the last 4 months.

Physical Examination.—A few râles on deep inspiration at the base of both lungs. The râles disappeared quickly and the lungs were clear at the time of the determinations of the venous oxygen. There is distinct bulging of the precordium. No thrills. The heart is considerably enlarged to the right and left, 4 cm. to the right and 12.5 cm. to the left of the middle line of sternum. The thorax is small. The x-ray picture shows a considerable enlargement in both directions. The normal heart sounds are replaced by blowing systolic and diastolic murmurs over the precordium. In the pulmonic area the pulmonic second sound can be heard, besides systolic and diastolic murmurs. The heart action is regular and rapid. The pulse is regular and small, collapsing in type. Blood pressure: systolic 116, diastolic 65. The electrocardiogram shows regular heart rhythm and signs of hypertrophy of the left ventricle. He is moderately cyanotic, the superficial veins are distended, and he is dyspneic. Liver extends from the fifth rib to 10 cm. below the costal margin in the nipple line, and is tender. Spleen is not felt. No ascites. There is slight edema of the lower legs and more distinct edema posteriorly over the hips. The edema over the hips disappeared quickly. Diuresis small, 420 to 740 cc. In the urine is found a trace of albumin; no casts.

[3] See the discussion in Papers II and III.

Treatment.—He was confined to bed; had salt-free diet and restricted calories. From September 5 to September 10 he had digipuratum about 0.5 gm. a day, without any appreciable effect on the circulation. Besides, he had iron, arsenic,



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis.

and aspirin. His clinical condition as a whole was unchanged during the determination of the blood.[4] He ran some temperature at the time of determinations of the oxygen (rectal temperature between 100 and 101°F.).

[4] September 27. A determination of the blood oxygen (not given in the table) showed an unsaturation of 9 volume per cent. His condition was the same as before. October 21. He was discharged; his clinical condition was unchanged.

This is a case of incompensated valvular heart disease on rheumatic basis. He probably has some endocarditis too. The values for the oxygen unsaturation are all above the upper normal limit. They are rather constant, all being between 8 and 10 volume per cent. The clinical condition was as a whole constant and unchanged, although the edema showed a tendency to disappear. It is important to emphasize that he was running a little temperature (100–101° F.) all the time. It is possible that this may have some effect on the oxygen unsaturation on account of increased metabolism.[5] His lungs were perfectly clear. It is interesting to note the constancy of the values for the oxygen unsaturation compared with those found in the three following cases (fibrillators). It is more constant than the values found in the normal individual (Papers I and II). The margin of action of his heart is probably very limited.[6] Digitalis was without effect—a striking contrast to what was found in the three fibrillators.

Case 3 (Table III, Text-Fig. 3).—Z., housewife; age 54 years. Admitted June 21, 1917. Discharged July 25.

Diagnosis.—Chronic cardiac disease (auricular fibrillation); chronic myocarditis.

Previous History, Symptoms, etc.—Shortness of breath and swelling of legs for the last 3 months. The symptoms have continuously grown more and more severe. Neither rheumatic fever nor lues.

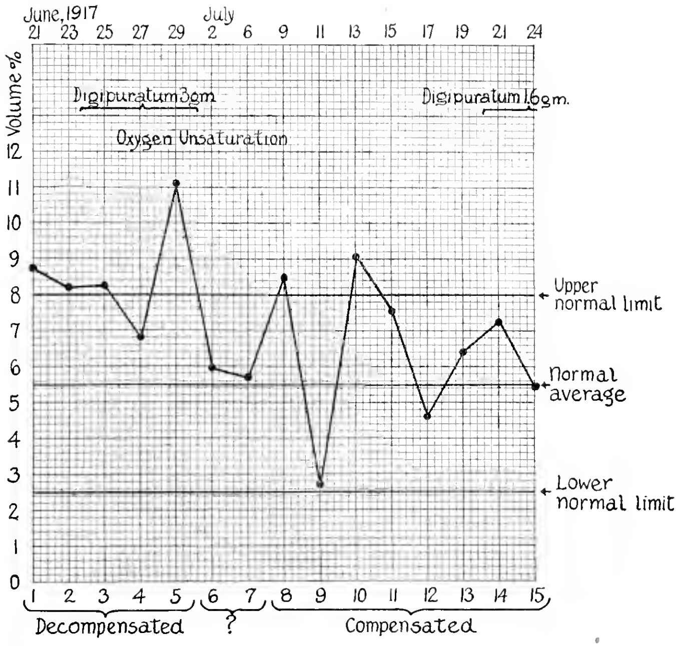
Physical Examination.—A few râles during the last part of the inspiration on both sides from the eighth to the tenth rib. No distinct dullness. There is marked dyspnea, slight cyanosis, and distinct swelling of the superficial veins. Heart dullness from 3 cm. to the right of the middle line of the sternum to 14 cm. to the left in the fourth intercostal space. X-ray picture shows the heart moderately enlarged to the left. The heart action is very rapid and irregular. The electrocardiogram shows auricular fibrillation. No distinct murmurs are heard. The aortic and pulmonic second sounds are distinct and equal. The patient is generally a fibrillator. Blood pressure: systolic 185, diastolic 120. It is difficult

[5] See the discussion in Paper II.

[6] As mentioned in Paper III, a valvular defect (aortic and mitral insufficiency, aortic stenosis) or increased blood pressure probably influences the action of the heart in the same way that a heavy wheel influences a machine. In mitral stenosis with auricular fibrillation the opposite holds true. The reason is that the increased resistance is here offered to the auricle instead of the ventricle (see the discussion on page 559, Lundsgaard (5), where the difference between the auricular and the ventricular form of heart failure is pointed out according to the result of the blood flow determinations reported in the paper referred to).

to decide whether or not the liver is enlarged. No tenderness over the left epigastric region. Spleen is not felt. No ascites. Wassermann test negative. Urine negative for albumin. Blood urea normal. Temperature normal.

Treatment.—She was confined to bed until July 14. From that date she was



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of chronic cardiac disease (auricular fibrillation); chronic myocarditis.

allowed to be out of bed, increasing the time every day. She had milk diet (800 cc.) for the 1st week. Since then salt-free diet (1,800 to 2,000 calories). Digipuratum, 0.5 gm. a day, was given from June 24 to 30 and about 0.3 gm. a day from July 20 to 25. She lost rapidly in weight from the 1st day in the hospital in spite of a rather small diuresis. Temperature normal.

TABLE III.

Case 3. Chronic Cardiac Disease (Auricular Fibrillation); Chronic Myocarditis.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	June 21	In bed.	13.83	13.84	13.83	122	22.57	8.74	96*/91	44†	+++	+	++	?	+++			66.1
2	" 23	" "	12.54	12.12	12.33	111	20.54	8.21	112/87	36†	++	+	++	?	+++	1,200	635	64.6
3	" 25	" "	11.85	11.43	11.64	107.5	19.89	8.25	110/102	36‡	++	+	++	?	++	1,300	820	62.6
4	" 27	" "	13.64	13.84	13.74	111	20.54	6.80	118/87	33	++	?	++	–	++	1,355	1,955	58.6
5	" 29	" "	9.70	9.86	9.78	113	20.91	11.13	89/83	38	++	+	++	–	+	1,500	990	56.3
6	July 2	" "	15.85	15.85	15.85	118	21.82	5.97	86/83	24	?	–	?	–	?	1,500	620	54.6
7	" 6	" "	16.76	16.06	16.41	120	22.20	5.79	82/82	28	–	–	–	–	?	1,300	815	54.1
8	" 9	" "	15.08	15.08	15.08	122	22.57	7.49	110/90	28	–	–	–	–	–	1,300	770	54.0
9	" 11	" "	19.48	18.80	19.14	118	21.82	2.68	100/72	28	–	–	–	–	–	1,150	965	54.0
10	" 13	" "	11.23	11.79	11.51	111	20.54	9.03	89/86	32	–	–	–	–	–	1,400	825	53.6

11	July 15	3 hrs.	12.10		12.10	106	19.62	7.52	100/92	28	—	—	—	—	—	1,300	980	52.6
12	" 17	1 hr.	14.66		14.66	104	19.25	4.59	?			—	—	—	—	1,300	800	53.0
13	" 19	1 "	12.82		12.82	(104)	(19.25)	6.43	92/68	30	—	—	—	—	—	1,300	400	53.2
14	" 21	1 "	11.26	11.02	11.14	100	18.50	7.36	94/82	30	—	—	—	—	—	1,300	450	52.8
15	" 24	2 hrs.	12.16	12.16	12.16	95	17.58	5.42	87/84	28	—	—	—	—	—	1,600	400	52.5

* The upper figure is the apex pulse, the lower the radial pulse.

† A few râles at the base of the lungs.

‡ The lungs were clear from June 25 on.

This patient was distinctly incompensated at admission, but recovered quickly (Table III). The values for the oxygen unsaturation of the venous blood started above the upper normal limit. At the time of the first two determinations the patient had râles in the lungs. At the third determination the lungs were clear and remained so during the rest of the time. In spite of the difference in the condition of the lungs no variations were encountered in the oxygen unsaturation of the venous blood of the first three determinations, a fact which supports what has previously been suggested (Paper II) that râles do not necessarily influence the oxygen unsaturation of the venous blood. This cannot mean anything else than that the saturation of the arterial blood has been as complete as in individuals with normal lungs. The fourth oxygen determination shows a value within normal limits; the clinical signs of incompensation were decreasing but still to be seen. The fact that a value for the oxygen unsaturation within normal limits can be obtained in a stage of clinical incompensation does not disagree with what has been previously found in Paper II.[7] This question will be discussed in the next case.

The fifth determination shows the highest values for the oxygen unsaturation obtained in this patient. The next day it went down to the normal average. At that time it was questionable whether she was clinically incompensated or not. On July 7 it was evident that the clinical symptoms of incompensation had disappeared and from that date the oxygen unsaturation as a rule was within the normal limits. In two instances, however, it was above the upper normal limit and as a whole it was variable. The fact that we can obtain values for the oxygen unsaturation above the upper normal limit in a period of full compensation agrees with the findings in Patient 5, in Paper III, and with those in the two following patients in this paper. It is probably explained by the fact that the values for the oxygen unsaturation of the venous blood are above the upper normal limit in the incompensated period and within normal limits in the compensated period. As in other fibrillators, however, we may

[7] In this publication all the determinations with compensated cases showed values within the normal limits, whereas all the values obtained in the incompensated cases were above. See the discussion in Paper II.

find abnormally high values in the period of compensation. The relation of the curve for the oxygen unsaturation to the digitalis treatment is unmistakable, and the same is found in the following patients and discussed more in detail.

Case 4 (*Table IV*, *Text-Fig. 4*).—D., housewife; age 37 years. Admitted July 19, 1917. Discharged October 14.

Diagnosis.—Mitral insufficiency and stenosis; auricular fibrillation.

Previous History, Symptoms, etc.—Palpitation and shortness of breath, particularly on exertion. Swelling of legs. She had rheumatic fever 14 years ago, but had no heart trouble at that time. Never had lues. For the last 2 years she has suffered from her present symptoms, periodically, not continuously. 3 weeks ago she left a hospital in fairly good condition, but her symptoms reappeared quickly and have steadily grown worse.

Physical Examination.—No cyanosis or dyspnea. There is swelling of the superficial veins. The heart dullness is considerably increased, extending from the fifth interspace, 6 cm. to the right and 14 cm. to the left of the middle line of the sternum. The x-ray picture shows the heart shadow correspondingly increased. No thrill. A harsh systolic murmur, replacing the first sound, is heard over the precordium and transmitted upward in the direction of the left axilla. An indistinct presystolic murmur is heard at the apex. The pulmonic second sound is accentuated and reduplicated. All the sounds at the bases are indistinct. The heart action is violent and irregular. The electrocardiogram shows auricular fibrillation. Systolic blood pressure 100 (palpation). Diastolic could not be determined. Liver is felt 10 cm. below the curvature in the nipple line; tender. Spleen not felt. No ascites. Marked edema extending upward over the hips. Urine negative for albumin. Wassermann test negative.

Treatment.—She was confined to bed and treated with milk diet (800 cc.) during the first 10 days. Later she had salt-free diet (1,800 to 2,200 calories). Besides iron, she had 1.6 gm. of digipuratum from August 22 to 26 (about 0.3 gm. a day). Later she had 1.6 gm. of digipuratum from August 27 to September 10 (about 0.1 gm. a day.) Her temperature was normal during all the determinations.

At the time of the first three determinations some râles were heard at the base of both lungs. Later on the lungs were clear. Thirty determinations of the oxygen unsaturation of the venous blood were done during two periods with an interval of 2 weeks between. In spite of the incompensated condition and the râles the values for the oxygen unsaturation were within normal limits the first five times. The first four determinations gave values which almost followed the normal average, the fifth was nearer the upper limit. The results

TABLE IV.

Case 4. Mitral Stenosis and Insufficiency; Auricular Fibrillation.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a—v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	July 19	¾ hr.	8.30	8.56	8.43	78	14.43	6.00	72/64	26*	—	—	++	+++	+++			68.4
2	" 20	In bed.	8.73	8.95	8.84	79	14.61	5.77	78/69	23*	—	—	++	+++	+++	1,750	333	
3	" 21	" "	9.51	9.51	9.51	82.5	15.28	5.77	89/80	20*	—	—	+	++	+++	1,025	280	
4	" 24	" "	8.33	8.73	8.53	78	14.43	5.90	72/70	20†	—	—	+	++	++	1,200	240	67.6
5	" 26	" "	8.25		8.25	82.5	15.28	7.03	66/60	22	—	—	+	++	+	1,200	820	67.8
6	" 28	" "	6.72	6.30	6.51	82.5	15.28	8.77	56/52	20	—	—	+	++	+	1,200	3,150	63.7
7	" 29	" "	11.16	10.72	10.94	84	15.53	4.59	44/44	20	—	—	?	++	+	1,200	2,825	51.2
8	" 30	" "	10.53	10.53	10.53	82.5	15.28	4.75	58/50	20	—	—	?	+	?	1,200	1,275	58.8
9	" 31	" "	11.17	11.17	11.17	82.5	15.28	4.11	48/48	20	—	—	—	?	?	1,200	630	57.2
10	Aug 1	" "	9.11	8.73	8.92	82.5	15.28	6.36	54/52	22	—	—	—	—	—	1,200	555	55.0
11	" 2	" "	9.17	9.15	9.16	87	16.10	6.94	52/52	24	—	—	—	—	—	1,200	525	54.1

Interval of 14 days.

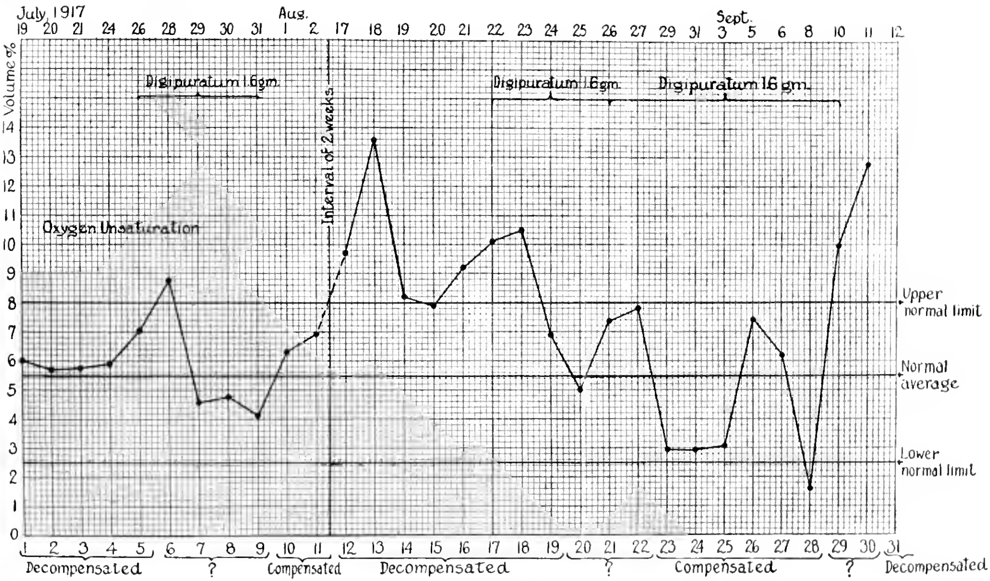
12	Aug. 17	In bed.	5.89	5.89	5.89	84	15.53	9.64	99/88	22	+	?	?	?	–	1,200	760	57.7
13	" 18	" "	1.97	2.02	2.00	84	15.53	13.53	96/92	24	++	+	+	+	?	1,200	730	57.7
14	" 19	" "	7.35	7.35	7.35	84	15.53	8.18	78/76	20	+	?	?	+	?	1,200	700	57.6
15	" 20	" "	7.40	7.84	7.62	84	15.53	7.91	78/60	20	+	?	?	++	?	1,200	615	58.2
16	" 21	" "	6.84	7.04	6.94	87	16.10	9.16	108/92	24	+	?	+	++	+	1,200	834	58.4
17	" 22	" "	6.05	6.05	6.05	87	16.10	10.05	120/84	24	+	+	+	++	+	1,200	555	58.7
18	" 23	" "	4.88	5.22	5.05	84	15.53	10.48	120/85	30	++	+	+	++	+	1,250	645	58.6
19	" 24	" "	8.55	8.13	8.34	82.5	15.28	6.94	98/88	24	+	+	?	+	+	1,200	993	59.2
20	" 25	" "	9.35	9.55	9.45	78	14.43	4.98	82/72	24	?	?	?	?	?	1,200	940	59.0
21	" 26	" "	7.16		7.16	78	14.43	7.27	?		–	–	–	?	?	1,200	1,280	59.1
22	" 27	" "	6.34	6.54	6.44	77	14.22	7.78	68/60	22	–	–	–	–	–	1,200	1,130	59.0
23	" 29	" "	11.86	11.86	11.86	80	14.79	2.93	64/64	20	–	–	–	–	–	1,200	1,000	58.6
24	" 31	" "	12.35		12.35	82.5	15.28	2.93	65/63	22	–	–	–	–	–	1,200	1,250	58.5
25	Sept. 3	" "	12.46	12.54	12.50	84	15.53	3.03	67/61	20	–	–	–	–	–	1,200	945	58.4

* Few râles.

† The lungs were clear from July 24 on.

TABLE IV—*Concluded.*

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a—v)	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
26	Sept. 5	In bed.	7.82	7.82	7.82	82	15.15	7.33	72/66	20	—	—	—	—	—	1,200	1,875	58.7
27	" 6	" "	9.02	9.02	9.02	82	15.15	6.13	76/72	20	—	—	—	—	—	1,200	610	59.0
28	" 8	" "	14.20	14.20	14.20	86	15.90	1.70	80/80	20	—	—	—	—	—	1,200	935	59.6
29	" 10	" "	5.87	5.87	5.87	85	15.72	9.85	72/59	22	+	+	+	?	—	1,200	1,355	60.0
30	" 11	" "	2.70	2.70	2.70	84	15.53	12.83	76/62	24	+	++	+	+	—	1,200	1,240	60.3



TEXT-FIG. 4. Oxygen unsaturation of venous arm blood. Case of mitral stenosis and insufficiency; auricular fibrillation.

of the first five oxygen determinations show the same things which were seen once in Case 3: a normal oxygen unsaturation at a time when the patient is distinctly clinically incompensated. This is probably an expression of the fact that she was improving. It will be seen from the chart that the severity of the symptoms of incompensation were decreasing. The fourth and fifth determinations showed an increasing tendency. Digitalis treatment was started on July 26. The oxygen unsaturation was still higher the next day (No. 6), this time above the normal limit. 1 day later a sudden drop in the oxygen unsaturation (4.6 volume per cent) occurred. Whether or not this was a result of the digitalis treatment cannot be definitely decided from the present facts. Nos. 8, 9, 10, and 11 were all within the normal limits. The oxygen determinations then had to be stopped for 2 weeks. At the time of the tenth and eleventh determinations she was clinically compensated; she felt well and was allowed to be out of bed.

This clinically compensated condition lasted for about 12 days. At that time (August 14) a rather sudden change in condition occurred. The patient began to feel uneasy. She had palpitations, throbbing in the veins of the neck, and pain in the left hypochondrium. On August 17 when the oxygen determinations were taken up again she was slightly incompensated. The oxygen unsaturation (9.7 volume per cent) was above the upper normal limit. The next day it was still higher (13.5 per cent), and the clinical symptoms of incompensation were more marked. The two following determinations (Nos. 14 and 15) showed a considerable drop in the oxygen unsaturation which went down to the upper normal limit. However, an increase appeared again and the incompensation seemed to be growing. Digitalis treatment was therefore instituted (August 22). 2 days later a sudden drop occurred in the oxygen unsaturation, almost identical with what was seen at the time of the first digitalis treatment. The clinical condition now improved rapidly. On August 25 it was impossible to decide whether she was incompensated or not, and 4 days later she was again in full clinical compensation. On August 24 and 25 the same condition was encountered as at her admission: normal oxygen unsaturation despite incompensation. However, as can be seen from the table, the clinical incompensation is rapidly dis-

appearing. On August 26 the digitalis dose was decreased to 0.1 gm. a day, instead of 0.5 gm. For 10 days she felt well and was compensated in rest. On September 10 she began to feel uneasy again. Her pulse rate and pulse deficit increased, and signs of incompensation appeared. The digitalis was stopped September 11 for a week. Her clinical condition grew rapidly worse and on September 12 she was again incompensated. Corresponding to the change in the clinical symptoms, a sudden large increase appeared in the oxygen unsaturation, which by September 10 was 10 volume per cent and on September 11, 12.8, which is far above the upper normal limit. The oxygen determinations had to be stopped at that time. A week later she was given digipuratum in larger doses, 0.5 gm. a day, and the symptoms of incompensation disappeared again. The oxygen determinations in this patient show: (1) that normal values for the oxygen unsaturation of the venous blood may be found with râles in the lungs; (2) that normal oxygen unsaturation may be associated with full incompensation but improving clinical condition; (3) that an increase in the oxygen unsaturation to above the upper normal limit may precede the clinical symptoms of incompensation; (4) the results suggest the oxygen unsaturation as an indicator of the action of digitalis;[8] (5) great variations in the oxygen unsaturation are met with in this patient, as in previous fibrillators.

Case 5 (*Table V*, *Text-Fig. 5*).—D., male, paper maker; age 31 years. Admitted February 9, 1917. Discharged May 5.

Readmitted June 19. Discharged July 25.

Readmitted August 22. Discharged October 2.

Diagnosis.—Mitral stenosis and insufficiency; auricular fibrillation; diabetes mellitus; slight chronic interstitial nephritis.

Previous History, Symptoms, etc.—Shortness of breath for 3 years before his admission to this hospital. At 9 years of age he had rheumatic fever without heart symptoms. Denies lues. After the heart symptoms had first started 4 years ago they grew continuously worse. Besides shortness of breath and palpitation he often had swelling of the lower extremities. He has been admitted to the hospital several times (four) in an incompensated condition and every time it has been more difficult, and taken longer to obtain compensation. Be-

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[8] The relation of the pulse rate and pulse deficit to the oxygen unsaturation suggests indirectly the same thing.

sides his heart disease he had a mild, not progressive diabetes and slight chronic interstitial nephritis.

Physical Examination.—The local physical signs of heart disease remained constant. At the first admission he had a considerable enlargement of the heart dullness 15.5 cm. to the right of the middle line of the sternum and 17.5 cm. to the left in the fifth intercostal space. No thrill. The x-ray picture showed corresponding enlargement of the heart shadow. At the apex a systolic and a diastolic murmur were heard. A systolic murmur was heard at the bases and the pulmonic second sound was accentuated. The pulse was irregular. The electrocardiogram showed arrhythmia perpetua. Blood pressure: systolic 180; diastolic 140. Wassermann test negative. In urine a trace of albumin, hyaline, and granular casts. Blood urea normal. Index of urea excretion fairly normal; now and then somewhat diminished. On ordinary mixed diet he would show sugar in the urine and about 0.20 per cent sugar in blood.

Treatment.—He was treated with digipuratum, antidiabetic and salt-free diet. Until he was absolutely compensated he was confined to bed. Now and then he had a slight increase of temperature (100–100.5°F.).

Forty-eight determinations of the oxygen unsaturation were done at three different periods, at the three successive admissions.

Period I.—Two determinations were done (February 17 and March 8). He had at that time been treated and the symptoms of incompensation had disappeared and he was fully compensated at rest. The values were normal (4 and 5 volume per cent).

Period II.—On June 19 he was readmitted to the hospital in a severely incompensated condition (dyspnea, cyanosis, distended veins, swelling of liver, and edema, as shown in Table V). Some râles were heard at the base of the lungs at that time. The temperature was slightly increased (100–102° F.) on the first 2 days; later normal. The determinations of the oxygen unsaturation showed considerably increased values. The first eight (the 1st week in the hospital) are all above the upper normal limit. He was at that time incompensated but improving. At the time of the first seven determinations (Nos. 3 to 9) moist râles were heard at the base of both the lungs. At the time of the eighth determination (No. 10) his lungs were clear. No difference is seen in the value for the oxygen unsaturation. The next four determinations (Nos. 11 to 14) show varying values—two above and two below the upper normal limit. A question mark on the diagram indicates that it was difficult to decide whether the patient was clinically compensated or

TABLE V.

Case 5. Mitral Stenosis and Insufficiency; Auricular Fibrillation; Chronic Interstitial Nephritis.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition	Sample 1	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	First admission.																	
	1917		*vol per cent*	*vol per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Feb. 17	In bed.	17.15	16.90	17.03	114	21.10	4.07	?/67	26*	—	—	—	—	—	1,600	948	64.7
2	Mar. 8	1 hr.	14.62	14.42	14.52	106	19.62	5.10	?/90	22*	—	—	—	—	—	1,900	1,200	71.1
	Second admission.																	
3	June 20	In bed.	4.69	4.29	4.49	88.8	16.42	11.93	93/88	24†	++	++	++	++	+++	1,000	525	72.0
4	" 21	" "	7.87	7.41	7.64	103	19.06	11.42	?/108	28†	++	++	++	++	+++	1,000	1,105	72.2
5	" 22	" "	10.34	10.34	10.34	100	18.50	8.16	?	—†	++	++	++	++	++	1,000	1,765	71.8
6	" 23	" "	8.15	9.07	8.61	101	18.70	10.09	?/72	28†	++	++	++	+	++	1,000	1,243	68.8
7	" 24	" "	8.57	8.50	8.54	100	18.50	9.96	77/76	24†	++	+	++	+	+	1,100	1,650	66.0
8	" 25	" "	11.85	11.85	11.85	105	19.94	8.09	78/72	26†	++	+	+	?	+	1,100	1,760	64.0
9	" 26	" "	9.14	9.14	9.14	101	18.70	9.56	80/70	26†	++	+	+		+	1,100	1,485	62.4

10	June 27	In bed.	8.21	8.21	8.21	101	18.70	10.49	71/70	28*	++	+	+	?	?	1,100	1,385	60.4
11	" 28	" "	13.44	13.24	13.34	108	19.96	6.62	68/65	22*	+	?	?	—	?	1,100	1,840	60.8
12	" 29	" "	6.20	6.18	6.19	106	19.62	13.43	?	—*	+	?	—	—	?	1,100	902	58.9
13	" 30	" "	11.65	14.65	14.65	111	20.52	5.87	?	23*		—	—	—	—	1,100	1,090	58.9
14	July 1	" "	11.34	10.92	11.13	112	20.72	9.59	75/66	21*		—	—	—	—	1,100	859	58.4
15	" 2	" "	12.72	12.42	12.57	116	21.45	8.88	69/65	25*		—	—	—	—	1,100	785	58.0
16	" 3	" "	15.56	15.56	15.56	116	21.45	5.89	68/74	24*		—	—	—	—	1,400	960	57.6
17	" 5	" "	10.54	11.48	11.01	116	21.45	10.44	69/61	28*		—	—	—	—	1,400	1,080	57.6
18	" 7	" "	15.42	16.60	16.01	116	21.45	5.44	59/57	28*		—	—	—	—	1,400	1,880	57.3
19	" 9	" "	9.00	9.00	9.00	116	21.45	12.45	78/66	28*	As a rule dyspnea only on exertion.	—	—	—	—	1,400	1,950	57.4
20	" 10	3 hrs.	9.38		9.38	117	21.64	12.26	72/67	28*		—	—	—	—	1,400	1,820	57.2
21	" 12	O.N.‡	17.50	17.50	17.50	117	21.64	4.14	66/66	26*		—	—	—	—	1,400	1,620	57.2
22	" 13	"	15.55	15.77	15.66	111	20.52	4.86	64/64	26*		—	—	—	—	1,400	1,140	57.2
23	" 14	"	9.91	9.01	9.46	104	19.25	9.79	68/64	26*		—	—	—	—	1,400	840	57.2
24	" 16	½ hr.	14.08	14.52	14.30	106	19.62	5.32	78/77	30*		—	—	—	—	1,400	1,035	57.4

* Lungs clear.

† A few râles at the base of the lungs.

‡ O. N. (over night in bed) means that the blood was drawn in the morning; ½ hr. means that the patient had been in bed ½ hour before the blood was drawn.

TABLE V—*Concluded.*

Determination No.	Bleeding: Date	Bleeding: Condition	Oxygen content of venous blood: Sample 1	Oxygen content of venous blood: Sample 2	Oxygen content of venous blood: Average (v)	Hemoglobin (Palmer's method)	Calculated oxygen capacity (a)	Oxygen unsaturation (a−v)	Clinical notes: Pulse	Respirations	Dyspnea	Cyanosis	Swelling of superficial veins	Swelling of liver	Edema	Fluid intake	Diuresis	Body weight
	1917		vol. per cent	vol. per cent	vol. per cent	per cent	vol. per cent	vol. per cent								cc.	cc.	kg.
25	July 18	1 hr.	16.56	16.52	16.54	106	19.62	3.08	80/72	30*	As a rule dyspnea only on exertion.	—	—	—	—	1,400	1,140	57.5
26	" 20	1 "	13.32	13.32	13.32	106	19.62	6.30	?	—*		—	—	—	—	1,400	1,415	57.4
27	" 23	O.N.	9.46	9.46	9.46	105	19.44	9.98	?/86	24*		—	—	—	—	1,400	760	57.4
28	" 24	1 hr.	13.67	13.42	13.55	105	19.44	5.87	88/86	32*		—	—	—	—	1,400	530	57.7
									Third admission.									
29	Aug. 22	$\frac{3}{4}$ hr.	11.08	11.08	11.08	111	20.52	9.44	88/78	38§	+++	+++	+++	++	+++			
30	" 22	In bed.	7.66	8.08	7.87	(111)	(20.52)	12.65	100/92	36§	+++	+++	+++	++	+++			
31	" 23	" "	10.02	10.02	10.02	110	20.36	10.34	100/78	32§	+++	+++	+++	++	+++	1,500	515	70.8
32	" 23	" "	8.92	8.92	8.92	(110)	(20.36)	11.44	108/98	34§	+++	+++	++	++	+++			
33	" 24	" "	10.17	10.53	10.35	110	20.36	10.01	96/88	44§	+++	+++	++	++	+++	1,500	680	71.4
34	" 25	" "	8.50	9.30	8.90	110	20.36	11.46	94/92	30§	+++	+++	++	++	+++	1,500	665	70.6
35	" 26	" "	8.96		8.96	106	19.62	10.66	89/74	28§	++	+++	++	++	+++	1,500	2,475	69.0

36	Aug. 27	In bed.	11.46		11.46	106	19.62	8.16	72/68	28§	++	+++	++	++	++	1,500	2,100	67.8
37	" 28	" "	9.38		9.38	107.5	19.88	10.50	160/80	28	++	+++	++	++	++	1,075	1,344	
38	" 29	" "	12.07	12.07	12.07	107.5	19.88	7.81	97/62	32§	++	++	++	++	++	1,400	315	
39	" 30	" "	12.30	11.80	12.05	107.5	19.88	7.78	74/68	32§	++	++	++	++	++	1,600	485	64.4
40	" 31	" "	4.57	4.57	4.57	107.5	19.88	15.31	67/63	32§	++	++	++	++	++	1,600	750	64.7
41	Sept 1	" "	12.65	12.65	12.65	107.5	19.88	7.23	68/64	28§	++	++	+	+	++	1,500	1,665	64.4
42	" 4	" "	4.92	4.68	4.80	106	19.62	14.82	76/64	32§	++	++	+	+	++	1,500	522	62.3
43	" 5	" "	11.52	11.72	11.62	106	19.62	8.0	74/66	30§	+	?	?	+	++	1,500	1,375	62.7
44	" 6	" "	7.67	7.67	7.67	106	19.62	11.95	78/70	32§	+	++	?	+	+	1,500	1,890	63.4
45	" 7	" "	11.25	11.25	11.25	103	19.06	7.81	73/70	28*	Only on exertion.	−	−	+	+	1,500	3,650	60.4
46	" 8	" "	14.00	14.00	14.00	104	19.25	5.25	78/64	24*		−	−	+	?	1,500	2,125	58.3
47	" 10	" "	13.00	13.00	13.00	104	19.25	6.25	72/66	24*		−	−	?	−	1,500	2,840	55.7
48	" 11	" "	11.86	11.92	11.89	104	19.25	7.36	74/70	24*		−	−	−	−	1,500	2,935	51.7

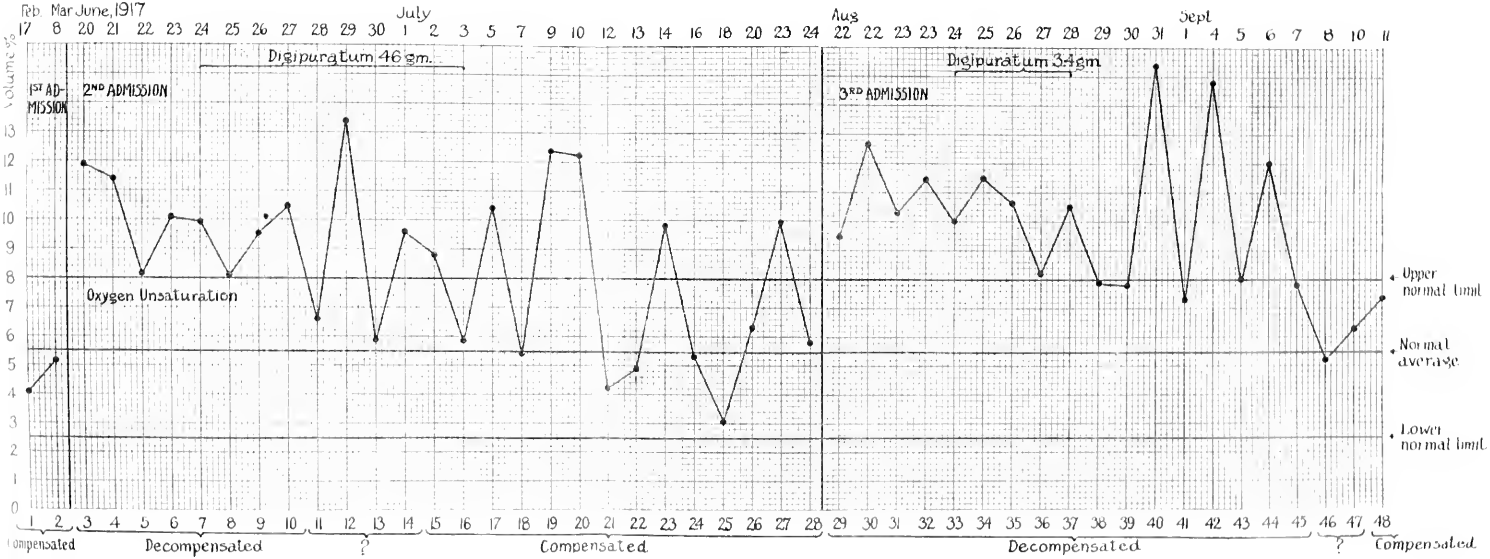
§ A few rather coarse râles at the left base; no distinct dullness.

not. On July 2 (determination 15) a period of clinical compensation at rest began. The values for the oxygen unsaturation show wide variations. Most of them are within the normal limits; a considerable number, however, are above the upper normal extreme. Upon the whole the values for the oxygen unsaturation are becoming lower and lower and there is a greater tendency to variations during the compensated than during the severely incompensated period. A line on the diagram shows the digitalis treatment (about 0.5 gm. of digipuratum a day from June 24 to July 3). It can be seen from the table that the edema decreased rapidly before the severity of the other symptoms of incompensation lessened. The same has been seen in other instances.

Period III.—A month later he was readmitted to the hospital. He had then for a week been in a condition of rapidly increasing incompensation which on admission was more severe than before. He was much more dyspneic and very cyanotic. The veins of the neck and arms were greatly distended and the edge of the liver was felt 10 cm. below the costal curvature in the nipple line. Heavy edema was noticed extending up his legs over the hips. The diuresis was small and he felt much distress. He was confined to bed and treated first with milk (800 cc. a day) for a week and later with salt-free diet (1,500 to 2,000 calories). From August 24 to 28 he had in all 3.4 gm. of digipuratum. It was then stopped on account of extrasystoles.[9] From September 1 to 3 he had 3 gm. of diuretin. The incompensated stage lasted this time longer than before (from August 22 to September 8). Seventeen determinations were done in this period. The first nine were all above the upper normal limit, occupying a rather limited space. The next ten were extremely variable, some of them going a little below the upper normal line; most of them, however, were far above.

His condition was only very slowly improving (Table V) until September 7 when a marked change took place. After 2 or 3 days

[9] The extrasystoles occurred regularly alternating with the ordinary ventricular beats. Only every other ventricular contraction was felt at the wrist. The apex rate was 160, the radial rate 80 (August 28, determination 37). The peculiar condition of the heart rhythm does not seem to have had any influence on the oxygen unsaturation of the venous blood (see Table V and Text-fig. 5).



TEXT-FIG. 5. Oxygen unsaturation of venous arm blood. Case of mitral stenosis and insufficiency; auricular fibrillation.

he became absolutely compensated at rest and felt extremely well. On the same date a considerable drop occurred in the oxygen unsaturation, which in the 5 following days (the last four determinations) was within normal limits. The oxygen determinations were stopped September 11. The patient was discharged October 2. He felt well and was clinically compensated. It will be seen from the table that râles were heard in the lungs from his admission (August 22) to September 6 (determination 44). From September 7 his lungs were clear. The first 4 days of his third admission he ran some temperature (100–101° F.). Thereafter his temperature was normal.

The values for the oxygen unsaturation in this patient agree as a whole with those previously found in patients with incompensated heart disease and auricular fibrillation. We can distinguish three periods in the course of the disease: (1) A period of severe incompensation. The values for the oxygen unsaturation are practically all above the upper normal limit, varying only to a moderate degree. (2) A transitory period where the symptoms of incompensation are disappearing or doubtful. In this period the values for the oxygen unsaturation are extremely variable, some above and some below the upper normal limit. (3) A period of clinical compensation at rest in which the oxygen unsaturation as a whole has a tendency to fall within the normal limits. Now and then, however, as seen in Patient 5, Paper III, the unsaturation exceeds the upper normal limit, a condition which never has been observed in compensated non-fibrillators.

DISCUSSION.

Relation of Oxygen Unsaturation to Clinical Symptoms.—As a whole, it can be said that the determinations of the oxygen unsaturation in the venous arm blood are of value in the diagnosis and treatment of patients with symptoms of circulatory disturbances. There is undoubtedly a close relation between the extent of the oxygen unsaturation and the clinical condition of the patients. As a whole, we have seen that the oxygen unsaturation in patients with compensated heart disturbances has values within the limits found in normal individuals, whereas patients with incompensated heart lesions show values above the upper normal extreme.

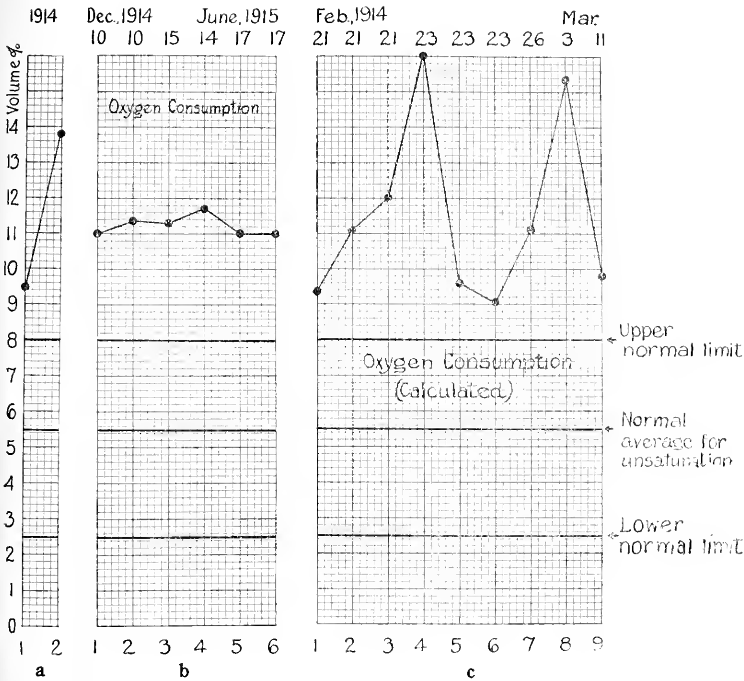
In patients in whom the incompensated condition of the circulation is decidedly improving we may find normal values for the oxygen unsaturation preceding the compensated stage, and we have seen an increase in the oxygen unsaturation to above the upper normal limit occur where incompensation was developing, but not yet clinically distinct.

In patients with auricular fibrillation the oxygen unsaturation may oscillate widely and show on some days values above the normal maximum, even when no incompensation is developing.

It is seen from the tables that the different clinical symptoms of incompensation differ in their relation to the oxygen unsaturation. Cyanosis and swelling of the superficial veins are closely related. Dyspnea appears to be usually accompanied in heart patients by increased oxygen unsaturation, but it is of course known also to occur in conditions, such as acidosis, in which the circulation is probably not retained. Edema seems to be to a great extent dependent upon special cases. It was usually possible to decrease the edema materially by the diet (salt-free) without interfering with the other symptoms and without causing any change in the oxygen unsaturation.

Oxygen Unsaturation and Blood Flow.—An attempt to calculate the rate of circulation (the minute volume of the heart) from the data obtained by the oxygen determination is not yet justified. Several facts, however, suggest that variations in the output from the heart have been by far the most important cause for the variations in the oxygen unsaturation in the patients. In Papers II and III a comparison was drawn between the directly determined oxygen unsaturation and the values for the oxygen consumption[10] calculated from blood flow determinations by means of Krogh and Lindhard's nitrous oxide method (5). A close parallelism was found, as far as the compensated patients were concerned,—even the peculiarities in the fibrillators were encountered by both methods. A similar close agreement is seen in patients with incompensated circulatory disturbance. Text-fig. 6, *a*, *b*, and *c* represents the calculated oxygen consumption in three patients with incompensation. It will be seen that all the values thus calculated on incompensated patients

[10] The difference between oxygen unsaturation and oxygen consumption is defined in Paper II.



Text-Fig. 6, *a*, *b*, and *c* (*a*). Case of aortic insufficiency and stenosis; mitral insufficiency; incompensated.[11] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method. The normal average and the normal extremes for oxygen unsaturation are indicated.

(*b*) Case of aortic and mitral insufficiency and stenosis; auricular fibrillation.[12] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

(*c*) Case of mitral stenosis and insufficiency; auricular fibrillation; incompensated.[13] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

[11] Patient 8, Lundsgaard (5), p. 537.

[12] Patient 2, Lundsgaard (5), p. 521.

[13] Patient 3, Lundsgaard (5), p. 524.

by the Krogh and Lindhard method are above the upper limit for the directly determined oxygen unsaturation in normal subjects. The patients in Text-fig. 6, *b* and *c* (Krogh and Lindhard's method) were fibrillators. The patient in Text-fig. 6, *c*, shows extremely variable values, but they are all above the upper normal limit. She was at the time of the determination in a continuously incompensated stage.

It seems, therefore, justifiable to expect that further investigations of the oxygen unsaturation in patients with different forms of circulatory and pulmonic disturbances may give us sufficient data to allow a conclusion concerning the relation of the oxygen unsaturation to the circulation.

The results on fibrillators suggest strongly that there is a qualitative difference in the dynamic pathogenesis of the heart failure in patients with normal rhythm of the heart and in patients with abnormal rhythm. This has been previously pointed out and discussed by the writer.[14]

Oxygen Unsaturation and Digitalis Action.—It seems promising to apply the oxygen determination to the study of the action of drugs, particularly digitalis, in the circulation. Cohn and Fraser have shown (6) that digitalis in a certain quantity will alter the T wave in the ventricular complex of the electrocardiogram, and have given us a valuable means of detecting the appearance of the local action of digitalis. The relation of the oxygen unsaturation to the digitalis treatment in the case reported here seems to indicate that the determination of the blood oxygen may be used as an indicator of the appearance of the effect of digitalis on the circulation as a whole.

SUMMARY.

1. A report is made of 103 determinations of the oxygen unsaturation of the venous blood of five patients with incompensated heart diseases.

2. Values for the oxygen unsaturation within normal limits were found only under two circumstances: (*a*) in a stage of full compensation, and (*b*) in a stage of incompensation where the symptoms were rapidly lessening.

[14] Lundsgaard (5), pp. 549–560.

3. Values above the upper normal extreme were met with under three circumstances: (*a*) during incompensation, (*b*) during compensation just before the clinical symptoms of incompensation had developed, and (*c*) at times in patients with auricular fibrillations in a condition of complete and stable compensation.

4. A comparison has been drawn between the directly found value for the oxygen unsaturation and the values for the oxygen consumption calculated from previous experiments by the writer on the blood flow (minute volume of the heart), in patients with similar clinical conditions. A close agreement existed.

5. It seems probable from our experience with patients under digitalis therapy that the oxygen unsaturation affords an objective criterion of the positive effect of the therapy.

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[15] An extensive bibliography is found in the first two papers of this series (1, 2).

THE FACTORS CONCERNED IN THE APPEARANCE OF NUCLEATED RED BLOOD CORPUSCLES IN THE PERIPHERAL BLOOD.

I. Influence of Procedures Designed to Increase the Rate of Blood Flow through the Blood-Forming Organs—Exercise and Nerve Section.

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Plate 7.

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During the winter of 1915–16, while developing a method for isolating the circulation through the tibia of the dog (1), two of us became interested in the active vasomotor control which the vessels of the marrow were shown to possess. At this time we undertook to determine what factors of coagulation (2) arose in the bone marrow, and in order not to complicate conditions physiological salt solution, Ringer's solution, and physiological salt solution containing small amounts of sodium oxalate were used as perfusion fluids. These solutions were delivered to the bone by means of a simple constant pressure apparatus and though usually oxygenated as thoroughly as is possible with such fluids, still it must be considered that they were relatively oxygen-free. Under the conditions of the experiments it was found at once that various characteristic marrow cells appeared in the effluent. Thus, nucleated red blood corpuscles, occasional myelocytes, and even at times megakaryocytes could be identified. Our attention was directed to the question of cellular egress by the observation that vasoconstriction, produced by the addition of small amounts of adrenin to the perfusing fluid, resulted in instant diminution of the number of cells leaving the marrow, and with the characteristic vasodilatation which concludes the reaction a large increase in the number of cells was noted. These findings paralleled those made

frequently in studies of the cellular composition of blood taken from the rabbit's ear during extreme vasoconstriction and during the succeeding vasodilatation. A difference existed, however, in the fact that in this case the vasoconstriction prevented the movement of cells from their place of origin, and this at once suggested the possibility that in the marrow there existed a formative tissue with a cellular product, and that the appearance of this product, the development of leukocytosis, or the appearance of nucleated red cells in the peripheral blood, depended to some extent on control exerted by these vasomotor nerves. This supposition has been found to be questionable but has been deemed worthy of mention as it has given the basis for our attack upon the problem in hand, since if vasomotor nerves controlled the output of cells from the bone marrow they must accomplish this result by increasing and decreasing the blood flow through the tissue, this being their only conceivable function. Our experiments have indicated nothing distinctive in regard to the vasomotor arrangements in the marrow vessels, nor do the anatomical investigations of Ottolenghi (3) indicate possibilities other than those ordinarily premised in considerations of vasomotor reactions.

Increasing experience with the isolated bone marrow circulation made it evident that it would be possible, making use of appropriate perfusion apparatus (4) and using hirudinized blood, to circulate the tibial marrow thoroughly and with rates of flow which could be provided exactly. This work has been pushed forward and has been controlled by every means at our command since at best it involved radical experimental procedures. Our attention has been focussed upon the nucleated red corpuscles and our first presentations will deal almost entirely with the appearance of these cells in the peripheral blood.

It was, as has been said, our idea that increased and decreased blood flow might have something to do with cellular retention and extrusion by the bone marrow. Some ground existed for this belief. Thus, the leukocytosis of exercise is a common observation and crises of normoblasts in various anemias have been ascribed to circulatory phenomena in the marrow. In the perfusion experiments there existed a method for instant increase and decrease of blood flow through the marrow. What measures could be adopted

to achieve similar and therefore controlling results in the intact animal? Two of these are discussed in this paper; namely, exercise and section of the vasomotor supply to the four limbs. These procedures have been carried out in normal animals and in animals rendered anemic and hyperplastic as to their blood-forming tissue by repeated hemorrhages. Hemorrhage and immediate saline infusion gave the third method for dealing with the problem. It would seem that nothing could be added to the literature of this last measure; yet no accurate studies exist of the time of appearance of nucleated red cells after hemorrhage and infusion, nor has the relation of the reaction to preexisting hyperplasia been studied. It has in the past been impossible to measure the extent to which blood flow through the marrow is increased by blood dilution after hemorrhage. This has been readily accomplished by means of the isolated marrow preparation. A later paper will present the results of these experiments together with a review of former work upon this phase of the subject.

We shall give a brief review of the conditions under which nucleated red corpuscles are found in the peripheral blood, with a discussion of the marrow hyperplasias in which nucleated red cells do not enter the circulation. In most instances organs delivering a secretion must pass it through a definite channel. Once formed, this secretion can be made usable only after passage down a duct. In the bone marrow, while it seems possible to localize clearly erythrogenic groups, it has been impossible to follow the physiological act of the delivery into the circulation of young or adult cells. The character of the sinusoidal spaces into which these cells must pass is not thoroughly understood. Van der Stricht (5), Dominici (6), and Brinckerhoff and Tyzzer (7), as a result of examinations of the marrow structure in the rabbit, believe that in this mammal the endothelial walls of the sinusoidal spaces are incomplete and that the circulation consequently wanders freely among the marrow cells. It would seem physiologically impossible to keep such a tissue intact were it not protected against changes in circulatory rate with extraordinary efficiency. This last condition does not exist, for we have found it possible to increase rate and volume of flow to an extreme degree without dislocating marrow cells. It is impossible to report fully as yet upon the injected marrow, but all our preparations indicate complete vas-

cular integrity in the marrow of the dog. If this is the case, all the circulating erythrocytes in this animal must at some stage in their development pass through a membrane.

Unfortunately there is no definite histological knowledge upon the marrow circulation in the dog or in man. One is inclined to believe that the facts in regard to the marrow vessels can be obtained only by careful injections, and yet even the studies upon the rabbit, which have been accepted as picturing the typical vascular arrangement in the mammal, have not been made by this method. It is obvious, therefore, that without definite knowledge of the moves which nucleated red cells must make to reach the blood stream no final division can be made of the conditions under which they appear. The following classification of nucleated red cell appearance is, therefore, based merely upon the most significant circumstances which seem to attend extrusion.

Nucleated Red Cells from Non-Hyperplastic Marrow under the Influence of Certain Poisons.

Ricin and Saponin.—In several papers Bunting (8) has given the blood picture and marrow condition in rabbits poisoned by saponin and ricin. With the former particularly, nucleated red cells appear within $5\frac{1}{2}$ hours,—before anemia is pronounced. They do not make their appearance until anemia has been produced, however, unless there is definite marrow vessel injury evidenced by hemorrhages into the tissue substance.

While nucleated red cells are found in the blood stream in chronic arsenic poisoning, phosphorus, chloroform, mercury, and benzene poisoning, their appearance is not accompanied by the acute changes emphasized in the case of the two substances first mentioned. As far as we can find it reported, nucleated red cell appearance in chronic or acute poisoning with any of the latter group occurs in the presence of anemia and may be considered due to the anemia and not to endothelial destruction or to breakdown of the actual marrow stroma.

Appearance of Nucleated Red Cells in Relation to Certain Leukocytic Reactions.—Ewing (9) mentions that fact that "severe leukocytosis in children or even in adults may draw a few normoblasts into the cir-

culation." Roger and Josué (10) found congestion and hyperplasia in the marrow 48 hours after the injection of *Staphylococcus aureus* into rabbits. At this time hyperplasia affected the nucleated red cells and it was not until later that myelocytic increase was prominent. Rieder (11) found nucleated red cells accompanying leukocytosis some time after bacterial injections. Sherrington (12) reports the same findings during high leukocytosis induced by sterile irritation of the skin and peritoneum. Morawitz (13) has held that the tendency of nucleated red cells to appear with posthemorrhagic leukocytosis argues against the fact that oxygen lack is the stimulus causing marrow hyperplasia after hemorrhage. Since there is no synchronism between the development of this particular leukocytosis and blast appearance his argument becomes of little value. Apparently any stimulus which causes intense hyperplasia of either group of marrow cells will result in extrusion of a few members of the other variety, possibly as a result of growth pressure alone. We must realize that growth in an enclosed space demands room and that the blood spaces, the marrow sinusoids, offer the most yielding means of egress. Timofejewsky (14) injected dogs and rabbits intravenously with large amounts of septic material and within 11 minutes found many normoblasts in the circulating blood, these cells appearing in the presence of the leukopenia which immediately followed the injection. Maximum numbers of normoblasts were found 2 hours after injection. Twenty-nine experiments on dogs and rabbits are found in this series. In all cases the animals were injected suddenly with large amounts of exceedingly putrid material, and while the interpretation is not one for which definite assurance can be given, since examinations of the marrow were not made, one is inclined to believe that large injections of toxic substance may act like saponin and ricin, causing acute degenerations of the marrow.

Careful discrimination must always be made between the conditions governing acute blast extrusion in the cases just discussed and those which may readily develop in the course of severe infections. Thus, acute rheumatic fever may be attended by severe anemia, moderate leukocytosis, and the appearance of normoblasts in the circulation. But in this case there exists merely another instance of normoblast appearance in secondary anemia.

Nucleated Red Cells in Anemias.

Secondary Anemia.—That nucleated red cells enter the blood stream after single large hemorrhages or after repeated small ones has long been known. We shall at a later date present more accurate data upon the time of appearance of these cells, alterations in this time caused by repeated bleedings with resulting marrow hyperplasia, and other features of the reaction. Hemorrhage and resulting anemia will not dislocate nucleated red cells immediately, as the injections of saponin and putrid material will. There is a latent period, usually of several days, before one can be sure that the cells found have really left the marrow and have not been in the circulation before the bleeding.

Primary Pernicious Anemia and Chlorosis.—In these diseases blast appearance is irregular and there seems but one fixed feature in it; namely, that crises, first emphasized by von Noorden (15) and by Neudörfer (16), precede periods of increased red count and physical betterment. In aplastic pernicious anemias no nucleated red cells occur; in other cases they may be extremely few and yet at autopsy an intensely hyperplastic marrow may be found. Blast appearance in anemias is ordinarily the expression of a vigorous effort to regain a lost position. The loss of these young forms into the circulation is, however, an expense and simply indicates an advantageous marrow reaction without being advantageous in itself. Stockard (17) has emphasized the fact that red cell formation demands a characteristic setting whether in bone marrow, liver, or spleen, and we shall show in a later paper how thoroughly the organism is protected against the loss of young forms from the marrow even in the presence of extreme hyperplasia. Hyperplasia alone then will not cause nucleated red cell extrusion, and this introduces another set of conditions which must be considered.

Nucleated Red Cells in Conditions Not Preceded by Blood Loss, Producing Hyperplasia and Polycythemia.

We need review only the literature of polycythemia and low barometric pressure in which examinations for blasts have been made. Zuntz, Loewy, Müller, and Caspari (18) made careful observations of their own blood and of the blood of animals in their Alpine expedition. With the exception of some ob-

servations of Müller's which merit specific discussion, they never found normoblasts, though polycythemia occurred promptly and characteristically. Similarly Douglas, Haldane, Henderson, and Schneider(19) in the Pike's Peak expedition mention no nucleated red cells, though polycythemia was pronounced.

Lastly, Loevenhart and his collaborators (20) have made accurate examinations of the effects of low partial pressures of oxygen with normal and with reduced barometric pressures. They obtained polycythemia together with marked marrow hyperplasia "Blood smears from animals showing increased blood counts after exposure to atmospheres poor in oxygen when stained with Jenner stain show a number of basophilic erythrocytes. This staining reaction is characteristic of newly formed erythrocytes." There is no mention of appearance of undoubted young forms, normoblasts or megaloblasts. Loevenhart's experiments made use principally of rabbits. It is worth mentioning at this time that these animals show normoblasts in secondary anemia produced by large hemorrhages much less easily than do dogs. One dog only appears in the series in question and there is no special note upon this particular feature of the blood picture.

Among other experiments carried out at low barometric pressure we may mention first those of Gaule (21). This investigator made observations upon blood films in two balloon ascensions. The elevation reached was not great—4,200 meters at most—so that the immediate effect of oxygen lack, if this is the stimulus of high altitude, could not have been intensely acute. Yet he reports blood films loaded with normoblasts and publishes photomicrographs to illustrate this fact. We are inclined with Zuntz (18) to regard the evidence presented by these photomicrographs as due to artefacts and question the results emphatically. Schauman and Rosenqvist (22), however, in a series of experiments in which they reduced pressure suddenly in a large bell glass under which animals could be kept, an apparatus lacking, it is true, most of the refinements with which Loevenhart safeguarded his more thorough experiments, were able in a few cases to obtain evidence of sudden blast extrusion. This did not occur in all cases, but in two instances at least, within 24 hours, they found many blasts in the blood of one rabbit and one dog when previous observation had shown none. It is, however, a noteworthy fact and one which will be discussed in a later paper that nucleated red cells have no increased tendency to appear in the blood in the presence of advanced hyperplasia. Indeed, the first reaction noted in dogs subjected to repeated bleedings may be disappearance from the blood of the ordinary low normoblastic content. Something more than hyperplasia is needed to dislocate these marrow cells.

It may be held in relation to the conditions now under discussion that the stimulus is applied more slowly than we are accustomed to see it in secondary anemias due, for instance, to sudden large hemorrhages. This is true of most observations made upon human beings at high altitudes, since in these cases the experiments are not usually carried to the danger point. And it is to be remembered

that Schauman and Rosenqvist (22) by sudden reduction of pressure with no other change in the condition of the animal did find blast extrusion in two cases. Loevenhart and his associates (20) found it impossible to keep animals at oxygen percentages below 7.0. They, however, brought several animals to this minimum, kept them there a week, and include them in their statement as to the blood morphology; *i.e.*, many bluish-staining forms, no mention of blasts. The polycythemia of low oxygen tension occurs with considerable rapidity. In these experiments maximum increase was never obtained in less than 1 week, but some increase was usually evident after 2 or 3 days. Oliver (23) found immediate increase on arrival at a high altitude, and his experience is borne out by others, notably Douglas, Haldane, Henderson, and Schneider (19), Viault (24), Mercier (25), Miescher (26), and Schneider and Havens (27). It requires, however, extraordinary and dangerous application of the stimulus operative in these cases to cause blast extrusion. Another feature to be emphasized is that these cells will appear as readily and as quickly with the marrow in normal formative equilibrium as in the presence of much larger volumes of hyperplastic tissue.

Two other sets of experiments deserve notice in this connection.

Reusch (28) attempted to localize stimulation of the marrow by means of what he designated as oxygen lack. In 2 hour periods daily over a long time he tied off one leg of a dog with a rubber tube, thus cutting off all the circulation, or a large part of it, to the femur and to the tibia of the leg in question, believing that he could secure marrow hyperplasia in these bones by this method. He failed, and failed again in an attempt to get new islands of blood-forming tissue in the liver by means of hepatic artery ligation and consequent deprival of oxygen. These experiments attempt to localize stimulus and effect, a feature which is unfortunately lacking in work upon the bone marrow because of the inaccessibility of the tissue. Had this crude attempt at isolation of the tissue succeeded, the nature of the stimulus to hyperplasia might be considered to have been limited at least to some degree. While at present it appears justifiable to hold to the idea that oxygen lack achieves this result directly, we are always confronted by the fact that it may be the secondary result of oxygen lack upon other tissues.

Müller (29) has made the only investigations which we have been able to find of blood taken directly from the nutrient vein of the tibia of the dog. He isolated this vein by removal of the fibula with as little hemorrhage and interference with respiration as possible. Under these normal conditions the blood so obtained agreed in cellular composition with blood from other parts of the body. Within an hour after severe hemorrhage he found many nucleated red cells in the nutrient vein blood. Similarly these cells were obtainable after 20 minutes' clamping of the nutrient artery and after rather prolonged general asphyxia produced by breathing a nitrogen- or a carbon dioxide-rich atmosphere.

Müller attributes the appearance of these blasts to oxygen lack. He seems to show definitely that local asphyxia results in dislocation of the cells in question. There is no indication, however, as to how this acute reaction is brought

about and the observations are unfortunately given without thorough evidence of care taken in controlling the work, the manner of investigating numbers of nucleated red cells, etc. There is also no evidence as to the degree of hyperplasia of the marrow which existed when the observations were made. These are all matters of importance and may lead readily to misinterpretations of phenomena.

Müller also took part in one of the Alpine expeditions headed by Zuntz, and advantage was taken of his method for getting nutrient vein blood in the course of this group of experiments. While, as we have said, their animals showed no blasts in the peripheral blood stream, the statement is made that the nutrient vein blood was full of these cells when examined alone. The belief is advanced that nucleated forms are being continually cast into the blood stream but that they are apparently unable to survive in this free swimming condition and promptly disappear—where or how is left for conjecture. We shall show in our experiments that at times it is easy by means of exercise to change the number of normoblasts found in blood films, and we are disinclined to believe the results quoted, in the absence of evidence as to the procedures gone through before the decision of blasts in the circulation was given.

EXPERIMENTAL.

The Effect of Exercise.

The first procedure adopted to test the effect of circulatory changes in the bone marrow upon the peripheral blood of the intact animal was that of exercise. It seemed safe to assume that exercise, since it increases the flow of blood through the other organs of the body, would increase the flow through the bone marrow as well. There is no direct means of testing the effect of muscular work upon blood flow through bones since the dissection involved inevitably destroys the possibility of exercising efficiently.

The leukocytosis of exercise has already been studied. Schulz (30) in a series of observations upon men found that an increase of from 1,000 to 4,000 leukocytes per c. mm. of blood would accompany vigorous muscular effort. This leukocytosis occurred simultaneously with the increased pulse and respiration rates induced by the muscular work and, according to Schulz, disappeared in the course of 15 minutes as the pulse and respiration returned to normal.

Burrows (31) in a study of leukocytosis associated with convulsions has recorded two control experiments in which he found leukocytoses of moderate degree and short duration following vigorous exercise.

Schneider and Havens (32) in a series of 36 experiments made upon 9 subjects found an increase in the number of white corpuscles following exercise, which

varied from 1,170 to 11,670 cells per c. mm. Similarly, Hawk (33) in a series of observations on athletes has found an increase in the number of leukocytes after exercise from 1,930 to 9,500 cells per c. mm. Zuntz and Schumburg (34) in their studies on soldiers obtained an average leukocytosis, following exercise, of 43 per cent.

Larrabee, Tileston, and Emerson (35), in studies on young men before and after a Marathon race of 24 miles, record the finding of an invariable leukocytosis which varied from 14,200 to 27,700 cells per c. mm. of blood. These observers also record the fact that no abnormalities in size, shape, or coloring of red cells, and no nucleated red cells could be found. With this exception none of the above writers record observations on nucleated red blood corpuscles, the type of cell with which we are primarily concerned.

Our experiments may be considered under three main heads: the effect of exercise (1) upon normal animals, (2) upon hyperplastic animals with large numbers of normoblasts in the peripheral blood, and (3) upon hyperplastic animals showing few or no normoblasts in the peripheral blood stream.

Before discussing our results we shall review briefly our methods of making blood examinations. In selected cases specimens were obtained by withdrawing blood from a superficial vein with a hypodermic syringe, but in the majority of cases blood was obtained by subcutaneous puncture. Dogs were used as subjects throughout this series of experiments.

The examination of each specimen consisted in counting the numbers of red and of white corpuscles per c. mm. of blood, and in making blood films. The red corpuscles were diluted with Hayem's solution; the white with a 4 per cent solution of acetic acid. The same pipettes and the same Thoma-Zeiss counting chambers were used throughout and all blood counts were made by two of us. The blood films were stained with Wright's stain, and were then examined with respect to the number of nucleated red corpuscles present. In each film 1,000 white cells were counted and the number of nucleated red cells which were observed during the process of this count was recorded. Let us assume, for example, that a certain dog gave a leukocyte count of 15,000 and that the film from this animal showed 10 normoblasts per 1,000 leukocytes. The number of normoblasts per c. mm. of blood would then be estimated as being 10 times 15, or 150. This method of estimating nucleated red cells has been used with reason-

ably satisfactory results by Timofejewsky (14), by Bunting (8), and by others, and we agree with Timofejewsky in finding it more satisfactory than any method of direct counting in a counting chamber.

The Effect of Exercise upon Normal Animals.—Many investigators have noted that the peripheral blood of a large percentage of dogs normally contains nucleated red cells. Our observations amply confirm this fact. In 26 observations on the ordinary dogs used in the laboratory, before experimental procedures had been instituted, we found an average of 227 nucleated red cells per c. mm. of blood, with a range of from 0 to 2,485 cells. Of these 26 animals, 8 on first examination showed no nucleated red cells at all.

TABLE I.

Normal Dogs.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
1	Aug. 31	10.50	8,624,000	24,400	466	
		11.12–11.17				Vigorous exercise, running out of doors.
		11.20	8,432,000	26,500	689	
2	Aug. 29	11.50	7,440,000	14,700	0	
		11.57–12.13				Vigorous running out of doors; animal well blown.
		12.15	8,592,000	18,200	0	

In carrying out an exercise experiment the animal to be studied was kept as quiet as possible before the experiment began. In the earlier work, both before and after exercise, one red and one white count and two blood films were made, all specimens being obtained by subcutaneous puncture. In later experiments at least two sets of counts and of films were made both before and after exercise; one set of specimens being obtained by subcutaneous puncture, the other set by withdrawing blood from a superficial leg vein. In some cases blood was also obtained by cardiac puncture.

After satisfactory specimens had been obtained the animal was exercised as vigorously as possible. A variety of modes of exercise was used. Among these were running in a treadmill, being towed by

a motorcycle, and running on a leash until the animal was well blown. Red and white counts and films, exactly comparable to those taken before exercise, were then secured, and the figures for all four observations compared. The results obtained in this way, as far as nucleated red cells are concerned, were remarkably uniform. Every dog observed, whose blood contained normoblasts before exercise, showed a slightly larger number of these cells after exercise. Some animals in whose blood no nucleated red cells were found before exercise displayed a few of these cells after vigorous muscular effort; other animals continued to show none. Typical examples of this result are shown in Table I.

The Effect of Exercise upon Hyperplastic Animals with Large Numbers of Nucleated Red Cells in the Capillary Blood.—Observations as to the effect of exercise upon normal dogs were followed by similar studies on a number of animals rendered hyperplastic by repeated hemorrhages of varying severity and frequency. During the course of development of hyperplasia the effect of exercise upon the animals was frequently tested. Exercise tests were made, at times a few hours after a hemorrhage, at other times days or even weeks after the last bleeding. Animals treated in this way pass through several phases in respect to the number of normoblasts which appear in the peripheral blood stream. Normal animals, showing a few normoblasts, may present a slight increase after the circulatory stirring up of exercise. In the 24 hours immediately following a large hemorrhage no significant increase in normoblasts occurs. Specimens taken before and at frequent intervals immediately after a hemorrhage either show no difference in nucleated red cell content, or show at best a slight increase after the hemorrhage, due in our opinion to a general stirring up of the circulation such as obtains in exercise.

But in the early stages of blood regeneration, evidenced by increasing red cell counts, normoblasts appear in larger numbers. Exercise increases the number of these cells in the capillary blood,—an increase practically in inverse proportion to the amount the animal has been allowed to move about before the specimens are taken. Later, as regeneration becomes complete, normoblasts largely disappear from the circulation, and at this stage few or none can be obtained by exercise.

It is evident, therefore, that exercise plus anemia will not bring

forth normoblasts, or else these cells would appear as a result of the exercise immediately after hemorrhage. Nor is exercise coupled with marrow hyperplasia capable of causing their appearance, for nucleated cells do not appear following exercise in the late stages of regen-

TABLE II.*

Hyperplastic Dogs Showing Large Numbers of Nucleated Red Cells.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1916					
3	Oct. 7					Bled 350 cc.
Weight	" 16					" 175 "
12.5	" 20	2.58	4,440,000	15,800	316	
kilos.		3.01–3.16				Hard exercise in treadmill.
		3.21	4,412,000	16,600	896	
	Nov. 10					Bled 175 cc.
	" 17	2.10	3,928,000	50,700	659	
		2.15–2.26				Exercise, running in the hall.
		2.28	4,184,000	51,300	1,539	
4	Oct. 2					Bled 180 cc.
Weight	" 6	10.55	2,860,000	11,000	385	
7.5		10.59–11.11				Very moderate exercise.
kilos.		11.12	3,104,000	18,400	1,177	
	Oct. 9	4.05	4,024,000	15,100	1,721	
		4.08– 4.23				Moderate exercise in treadmill.
		4.30	4,312,000	16,800	2,318	
5	Oct. 21					Bled 360 cc.
Weight	" 22	9.40	2,736,000	17,300	138	
8.9		9.45–10.04				Hard exercise in treadmill.
kilos.		10.06	2,800,000	22,500	247	
	Oct. 25	9.30	2,976,000	34,200	684	
		9.31– 9.46				Exercise in treadmill.
		9.50	2,872,000	43,600	1,024	

* In bleeding animals, in order to secure as large a reduction of cells as was possible with safety, withdrawals of blood and injections of salt solution were alternated in the same operation. The figures given in the tables expressing the size of the hemorrhages represent, therefore, a mixture of blood and of recently injected salt solution.

TABLE II—*Concluded.*

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6						
Weight	Jan. 5					Bled 710 cc.
20.5	" 16					" 1,075 "
kilos.	" 17	2.25	3,048,000	36,700	1,321	From superficial vein.
		2 30	2,448,000	34,100	920	Subcutaneous puncture.
Average..			2,748,000	35,400	1,120	
		2.34–2.46				Running out of doors; dog refused to run hard.
		2.55	2,078,000	38,000	1,596	From superficial vein.
		3.00	2,592,000	35,200	1,795	Subcutaneous puncture.
Average..			2,335,000	36,600	1,695	
	Feb. 2					Bled 1,160 cc.
	" 23					" 1,000 "
	Mar. 2	2.20	4,304,000	20,100	201	From superficial vein.
		2.25	4,280,000	20,800	145	Subcutaneous puncture.
Average..			4,292,000	20,450	173	
		2.27–2.47				Hard running out of doors.
		2 50	4,528,000	20,300	1,096	From superficial vein.
		2 52	4,320,000	26,100	1,096	Subcutaneous puncture.
Average..			4,424,000	23,200	1,096	

eration when hyperplasia is extreme. Exercise gives more cells when cells are already present, and in this condition only.

The figures in Table II are taken from dogs in the early stages of the hyperplasia following hemorrhage. We have many more figures to the same effect but these given suffice to prove the point; namely, that animals, possessing in their capillary blood fairly large numbers of normoblasts before muscular effort, show still more of these cells after the general stirring up of exercise.

It seemed possible, at first, that the increase in nucleated red cells resulting from exercise was perhaps due to an output of cells from the marrow tissue, that the speeding up of the circulation occasioned by vigorous muscular effort had caused a real crisis of blasts, compar-

able perhaps to the blast crises of anemia. But further study convinced us that this interpretation was wrong. In experiments in which samples of blood were taken from different sources before exercising the animals, an unequal distribution of nucleated red cells was occasionally found. A specimen obtained by subcutaneous puncture, for example, would contain relatively few normoblasts while a second specimen taken from a superficial vein within a minute or two would show a much larger number of the cells. After exercise the second subcutaneous specimen would show a marked increase in the number of nucleated red cells, a result completely in accord with our former experiments. But the second vein specimen, on the contrary, would

TABLE III.

Effect of Exercise on Distribution of Nucleated Red Cells.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
3	*1916*					
	Dec. 23	11.45	3,744,000	26,700	747	From superficial vein.
		11.50	3,616,000	27,100	336	Subcutaneous puncture.
Average..			3,680,000	26,900	541	
		11.55–12.17				Hard running out of doors.
		12.20	Lost.	24,600	565	From superficial vein.
		12.22	3,648,000	24,400	561	Subcutaneous puncture
Average..			3,648,000	24,500	563	

show fewer nucleated red cells than before exercise. An average of the observations before and of those after exercise would give practically the same figure. The observations made on Dog 3, shown in Table III, illustrate this point.

This suggested that probably the increase of normoblasts in the capillary blood following exercise, which at first sight seemed possibly a real crisis of blasts extruded from the marrow tissue, was in reality merely the result of a general stirring up of the circulation and of a consequent more even distribution of blood through the peripheral area. Further experiments supported this idea. We found that any procedure calculated to cause a considerable stirring up of the circulation, such, for example, as hemorrhage and infusion, would im-

mediately increase the number of blasts in the peripheral blood, provided that any had been observed at the beginning. The suddenness of the increase in all these cases weighed in favor of the view that the newly observed cells had been hidden away in deep lying vessels, possibly in the veins of the marrow itself, and, as a result of the increased rate of circulation incident to the hemorrhage and infusion, had been driven out into the open.

As the experimental animals became more hyperplastic and developed refractory periods in relation to the extrusion of blasts, periods in which practically no nucleated red cells could be found in the peripheral blood either before or after exercise, we became convinced that exercise serves only to give a true picture of the nucleated red cells already in the circulation, and has no influence whatever on their extrusion from the marrow tissue.

The Effect of Exercise upon Hyperplastic Animals Showing Few or No Nucleated Red Cells in the Peripheral Blood Stream.—After a time many animals become adjusted to periodic bleedings and pass into periods when few or no nucleated red cells can be obtained by subcutaneous puncture, no matter how vigorous an effort is made to stir them out. These refractory periods have a definite relation to the process of regeneration after hemorrhage. In general they occur just after a hemorrhage when the red count is low, and again when regeneration is almost complete. If the animal has become adjusted to frequent bleedings and a particular hemorrhage is not a severe one, the refractory period may last throughout the entire process of regeneration. If, however, the hemorrhage is severe, the early refractory period is followed by a true formative crisis of blasts which lasts from 1 to several days. The height of this crisis varies with the severity of the hemorrhage and with the degree of ability to resist normoblast extrusion, which the animal may have developed.

During these two refractory periods, when few or no blasts are to be found in the peripheral blood, exercise will not bring them out. The figures given in Table IV are taken from two hyperplastic animals in refractory phases and will serve to demonstrate this point. The first figures in Table IV (Dog 5) represent a refractory period occurring after a hemorrhage when the red count was low. The second set of figures (Dog 7) are taken from a young dog whose

marrow was presumably hyperplastic at the beginning. When normal this animal showed no nucleated red cells before exercise and 123 per c. mm. after exercise. After one bleeding and regeneration to normal, he showed the completely refractory period recorded in Table IV. Throughout our entire experience with this animal he showed a remarkable resistance to the extrusion of blasts.

Table V gives an almost complete picture of a single dog passing through the various periods into which we have divided our exercise

TABLE IV.

Hyperplastic Dogs in Refractory Periods.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leuko-cytes per c.mm.	Blasts per c.mm.	Remarks.
	1916					
5 Weight 8.9 kilos.	Oct. 21 Nov. 10 Dec. 14					Bled 360 cc. " 190 " " 230 " Animal showed many blasts during this period.
	" 19	4.15	3,456,000	23,100	0	
		4.20– 4.35				Exercise, running about building.
		4.38	3,128,000	24,600	0	
7 Weight 5 kilos.	Oct. 13					Bled 175 cc.
	" 24	9.40	5,064,000	19,500	0	
		9.42– 9.57				Hard exercise in treadmill.
		10.00	5,232,000	18,500	0	

experiments. Unfortunately this animal was not exercised before it was bled, so no figures can be given showing the reaction to exercise while yet normal. On January 5, the first experimental day, this animal was bled 710 cc. Before the bleeding two observations showed no normoblasts in the peripheral blood. After the hemorrhage, which reduced the red count from 5,696,000 to 3,831,000, eleven observations gave an average of 15 normoblasts per c. mm. This increase we believe to have been due to the stirring up of the circulation incident to the hemorrhage and infusion, and in this respect to be com-

TABLE V.

Effect of Exercise on Dog 6 during a Series of Hemorrhages and Recoveries.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6 Weight 20.5 kilos.	Jan. 5	11.25–11.50	5,696,000	14,700	0	Average of two observations.
		12.00				Bled 710 cc.; infused 860 cc.
		12.10– 5.15	3,831,000	25,920	15	Average of eleven observations.
	Jan. 16					Bled 1,075 cc.
	" 17	2.25	3,048,000	36,700	1,321	From superficial vein.
		2.30	2,448,000	34,100	920	Subcutaneous puncture.
Average..			2,748,000	35,400	1,120	
		2.34– 2.46				Running out of doors; dog refused to run hard.
		2.55	2,708,000	38,000	1,596	From superficial vein.
		3.00	2,592,000	35,200	1,795	Subcutaneous puncture.
Average..			2,650 000	36,600	1,695	
	Feb. 2					Bled 1,160 cc.
	" 23					" 1,000 "
	Mar. 2	2.20	4,304,000	20,100	201	From superficial vein.
		2.25	4,280,000	20,800	145	Subcutaneous puncture.
Average..			4,292,000	20,450	173	
		2.27– 2.47				Hard running out of doors.
		2.50	4,528,000	20,300	1,096	From superficial vein.
		2.52	4,320,000	26,100	1,096	Subcutaneous puncture.
Average..			4,424,000	23,200	1,096	
	Mar. 8	2.50	4,992,000	24,600	24	From superficial vein.
		2.54	4,976,000	25,300	25	Subcutaneous puncture.
Average..			4,984,000	24,950	24	
		2.55– 3.10				Running out of doors; animal well blown.
		3.15	5,912,000	24,000	336	Subcutaneous puncture.
		3.17	5,880,000	23,200	116	From superficial vein.
Average..			5,896,000	23,600	226	
	Mar. 13	2.18	6,152,000	20,200	0	From superficial vein.
		2.21	5,824,000	22,200	22	Subcutaneous puncture.
Average..			5,988,000	21,350	11	

TABLE V—*Concluded.*

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leuko-cytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6	Mar. 13	2.23– 2.38				Running out of doors.
		2.39	6,856,000	36,300	108	From superficial vein.
		2.40	6,616,000	32,200	96	Subcutaneous puncture.
Average..			6,736,000	34,250	102	
	Mar. 14					Bled 1,000 cc.
	" 17	8.35	4,072,000	27,860	55	From superficial vein.
		8.37	3,608,000	30,500	0	Subcutaneous puncture.
Average..			3,840,000	29,180	27	
		8.38– 8.55				Running out of doors.
		8.57	3,768,000	27,700	27	From superficial vein.
		8.58	3,448,000	26,500	53	Subcutaneous puncture.
Average..			3,608,000	27,100	40	

parable with an exercise increase. The figures on this animal for January 17, March 2, and March 8 show a typical reaction to exercise in the early stages of hyperplasia. The number of normoblasts in the animal's blood on these days was very high before exercise, and in each case was decidedly increased after exercise. It is interesting to note in this table the gradual development of an ability on the part of the animal to keep the nucleated red cells This tendency reaches its height, as far as this series of observations is concerned, on March 17, when the animal, starting with an average of 27 blasts per c. mm. before exercise, showed an average of 40 of these cells after exercise.

The Effect of Nerve Section.

In the early days of microscopic blood examinations, when the relative constancy in health of the red and of the white blood count was first appreciated and when the speed with which these counts could alter in disease became known, it was natural that investigators should look for nervous control of blood composition. Thus, Cohnstein and Zuntz (36) in 1888 examined the results of the complete vascular paralysis caused by spinal cord section at the level of the seventh cervical vertebra. They found that a great lowering of the red and of the white count followed this section, which was of course accompanied by complete vaso-

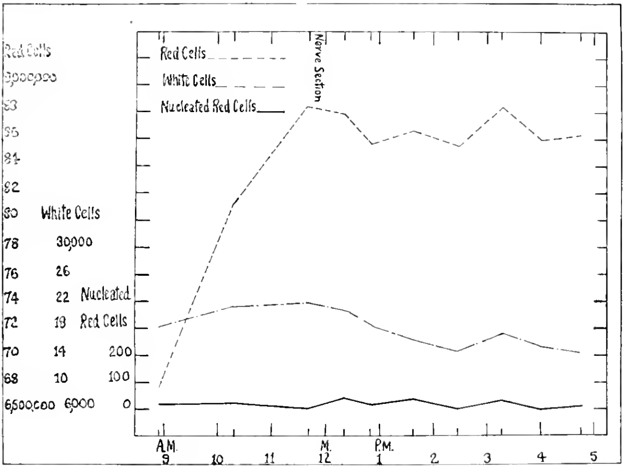
motor collapse. They attributed this blood dilution to transudation of tissue fluid into the vessels when their tone was destroyed. Malassez (37) in a brief note states that denervated parts usually give lowered red counts, but he presents no more than this brief statement.

It is obvious that such experiments as those of Cohnstein and Zuntz would not achieve the result demanded by our experiments, namely, increased flow through the marrow vessels, since in the collapse following complete vasomotor palsy the peripheral blood stream becomes very sluggish. It was therefore necessary to provide nerve sections which could be shown to have no effect on general blood pressure but which would at the same time open a large number of marrow vessels. We therefore cut the two sciatic nerves very high in the thighs and both brachial plexuses above the upper ends of the humeri.. These sections result in vasomotor paralysis of the vessels in the long and the small bones of the four limbs. The operation in each experiment was carried out under sterile conditions. Blood examinations (erythrocyte, leukocyte, and normoblast counts) were made prior to surgical procedure. All operations were performed under morphine and cocaine anesthesia with brief administration of ether, if necessary, at the time of the nerve sections. Blood pressure tracings were made before nerve section and afterwards at intervals throughout the remainder of the day in order to follow the circulatory condition of the animal. The operation never gave shock. Following the nerve section we were able to maintain a normal blood pressure throughout the rest of the day while we followed at frequent intervals the cellular composition of the blood in order to see whether the increased blood flow through the bones enumerated caused egress of cells.

The results of the nerve section experiments were negative. There occurred either no change at all in the number of normoblasts present in the peripheral blood, or else so slight a change as to be within the limits of error of the method used in counting the cells.

Text-fig. 1 gives a graphic picture of a typical nerve section experiment performed on a normal dog (Dog 8). Three observations on this animal before nerve section, extending from 8.55 a.m. to 11.41 a.m. gave an average of 13 nucleated red cells per c. mm. of blood; seven observations after nerve section, extending over a period of 5

hours, gave an average of 21 nucleated red cells per c. mm.—an insignificant increase. The early rise in the erythrocyte count shown in this chart is a phenomenon, rather marked in this case, which we have often seen following morphinization. Before nerve section, however, the red count had reached a level which remained more or less constant throughout the afternoon.



TEXT-FIG. 1. Curve showing the absence of effect of section of nerves to the four limbs upon the red cell, the white cell, and the nucleated red cell counts in Dog 8. The ordinates represent cells per c. mm. of blood; the abscissæ represent time of day. The initial rise in the red cell count before nerve section is due to morphinization.

Fig. 1 gives the blood pressure record of the same animal during the course of the experiment. Tracing 1 was made shortly before section of the nerves. Tracing 2 was made before, during, and after nerve section, the time occupied by nerve section being indicated by two crosses (+ .... +) on the record. Subsequent tracings, 3 through 9, were made at intervals during the 5 hours following nerve

section. It will readily be seen that the animal maintained a normal blood pressure throughout the course of the experiment.

A similar experiment was performed on Dog 7 which had been bled and infused five times previously and whose marrow as seen at autopsy showed extreme red cell hyperplasia. Two observations on this animal before nerve section gave an average of 41 nucleated red cells per c. mm.; eleven observations, during a period of about 5 hours following the operation, gave an average of 47 nucleated red cells per c. mm. In this animal $3\frac{1}{2}$ hours after section of the nerves the effect of general asphyxia upon normoblast extrusion was tested by repeated clampings of the trachea. This procedure caused each time a marked rise in blood pressure but had no effect upon the appearance of nucleated red cells in the peripheral blood.

With the exception of one animal (Dog 9), in which the small but decided increase after nerve section of from 645 (average of three observations) to 908 (average of seven observations) normoblasts per c. mm. was obtained, all nerve section experiments gave negative results.

CONCLUSIONS.

1. Increase in circulatory rate caused by hard exercise has no power to dislocate nucleated red cells from the bone marrow either in normal, in anemic, in hyperplastic, or in anemic and hyperplastic animals.

2. In anemic and hyperplastic animals pseudocrises of nucleated red cells can be produced at certain periods by hard exercise, but careful analysis leads inevitably to the conclusion that the increase in these cells is merely a more accurate expression of circulatory content at the time of the procedure.

3. Section of the vasomotor nerves to the four limbs with consequent dilatation of the marrow vessels and increased blood flow through the tissue does not result in the freeing of nucleated red cells from the bone marrow.

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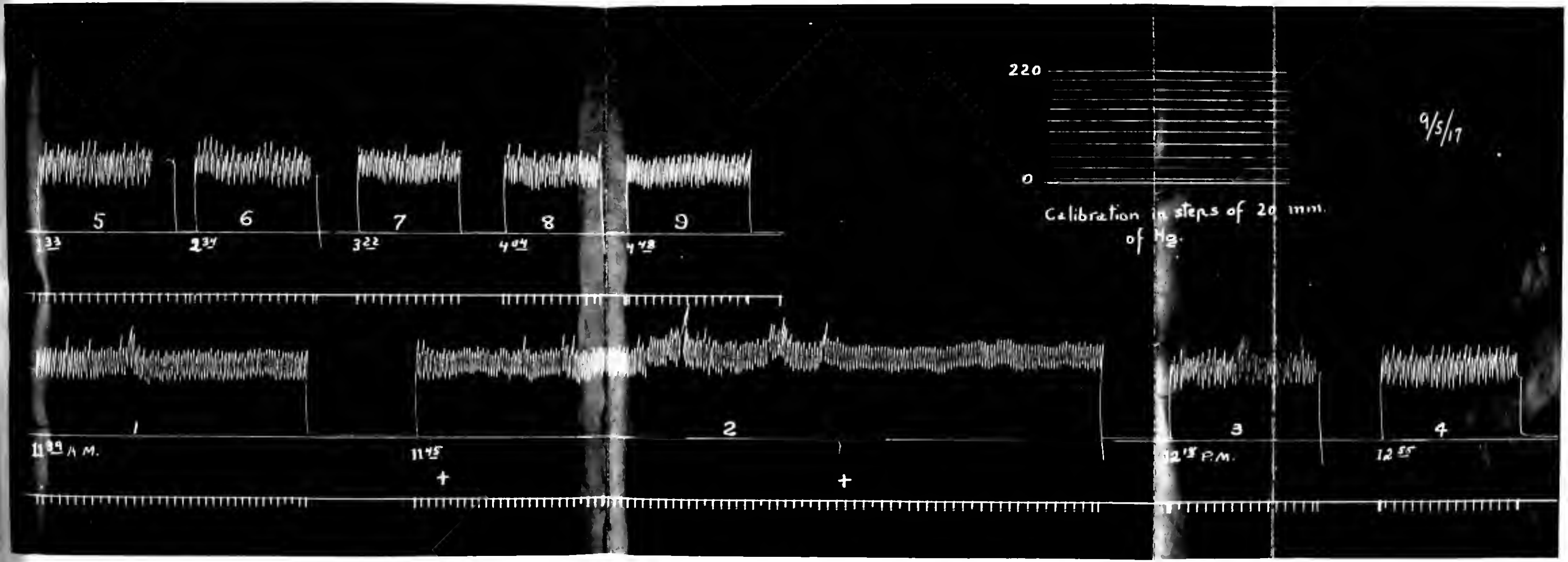
EXPLANATION OF PLATE 7.

FIG. 1. Graphic record of the arterial blood pressure of Dog 8 taken at intervals during a period of 5 hours preceding, during, and following section of nerves to the four limbs. Nerve section, performed during the recording of tracing 2, caused no immediate or subsequent fall in general arterial pressure.


220
0
Calibration in steps of 20 mm. of Hg.
9/5/17
5
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2
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4
11 39 A.M.
11 45
12 18 P.M.
12 55

Fig. 1.

SKIN FERMENTS.

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(Received for publication, September 7, 1917.)

During the past few years, particularly in connection with observations on the luetin reaction, the typhoidin reaction, and related bacterial skin tests, as well as with certain skin tests made with a variety of proteins in clinical conditions apparently dependent on a sensitized state of the patient (asthma, urticaria, etc.), greater interest has been stimulated in the study of the possible mechanism of these reactions and their relation to antibody as well as ferment concentration of the reacting tissues.

Kolmer (1) has reviewed the subject in a recent paper. He calls attention to the fact that, aside from the sharply specific reactions, certain non-specific and as yet unknown factors play a part in some of the skin reactions. The work that has followed the observations of Sherrick (2) concerning the part the iodides play in influencing skin reactivity has emphasized this point of view. With these considerations in mind we have undertaken a study of the enzymes of human skin and of skin from several of the common laboratory animals.

Phylogenetically we might expect that the skin would elaborate ferments to a greater degree than most other tissues, since the ectoplasm of the cell (and the differentiated ectoderm) apart from its protective function is that portion of the cell brought into relation with the surrounding medium and therefore intimately associated with any secretory mechanism designed to alter food particles through extracellular digestion. Of course, with the continued differentiation into a protective structure we may assume that the ferment secretory activity would be repressed. As far as the literature is available to us we have found but few references to work dealing with skin ferments.

Material.—The skin material was prepared as follows: The skin was stripped from the underlying tissue from freshly killed animals, and from fresh autopsy material in the case of the human skins. The animals were not exsanguinated, so that the normal amount of blood remained in the corium. With a razor as much of the connective tissue of the corium as possible was then removed, leaving the flaps practically free from connective tissue. The skin was next cut into small bits with sharp scissors, spread on a glass plate, and rapidly dried in an air current at low temperature (25°C.). The dried material was then placed in a desiccator over night and ground the following morning. The powdered skin was put through a 40 mesh sieve. The dried powder was made up as a 0.5 per cent suspension in isotonic salt solution for use.

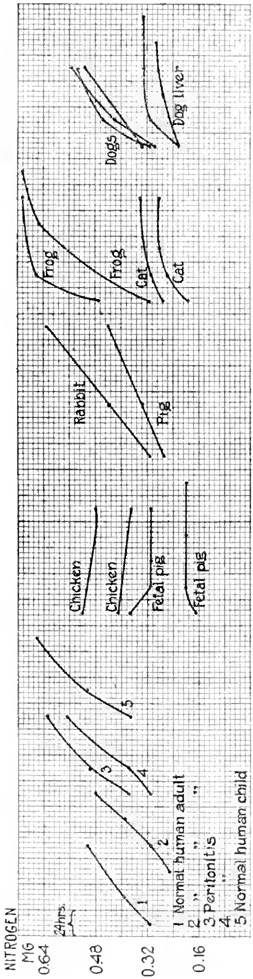
Inasmuch as the antigens ordinarily employed in skin reactions are largely protein in character, we have endeavored to study the proteolytic processes of the skin in greater detail, including (*a*) the simple autolysis of the skin (without altering the reaction); (*b*) digestion of a foreign protein (casein); (*c*) the relative resistance of the skin to tryptic digestion; (*d*) the peptidase content of the skin.

Autolysis of the Skin.—The skin suspensions were autolyzed for several days under toluene at 45°C. At definite times, 24, 48, 72 hours, etc., 2 cc. samples were withdrawn and the amount of non-coagulable nitrogen was determined. The relative rate of autolysis is illustrated in Text-fig. 1.

The human skins all autolyzed at practically the same rate and evidently to an extent equal to that of the animal skins. On the other hand, the skin of the young animals (fetal pig, young chicken) gave no evidence of autolysis, while of the skins of the adult animals that from the frog autolyzed to the greatest extent. For purposes of comparison the autolysis of the liver, dried with its contained blood, is shown in the last curve of Text-fig. 1.

Protease.—In order to study the possible liberation of proteases from the skin two experiments were made. In the first, 1 cc. each of the skin emulsions was incubated 48 hours with 2 cc. of a 1 per cent casein solution made slightly alkaline to phenolphthalein with sodium carbonate.

No alkaline active protease was demonstrable by this means. In



TEXT-FIG. 1. The relative rate of autolysis of skin suspensions from human subjects and animals.

the following experiment the casein solution was prepared slightly acid to phenolphthalein, and in this experiment the digestion of the casein proceeded as indicated in the following table:

	Nitrogen. mg.		Nitrogen. mg.
Liver	0.57	Rabbit	0.20
Normal human	0.3	Chicken	0.11
" dog	0.29	Pig	0.00
" "	0.11	Cat	0.00
" "	0.09	Frog	0.00

Resistance to Tryptic Digestion.—2 cc. of the skin suspension were incubated for 4 hours with $1\frac{1}{2}$ trypsin units. 1 unit digests 2 cc. of a 1 per cent casein solution in 1 hour. For purposes of control similar tubes were prepared and immediately boiled. The amount of the increase in non-coagulable nitrogen as a result of the digestion is shown in the following table:

	Nitrogen. mg.		Nitrogen. mg.
Dog	0.23	Pig	0.00
"	0.07	Fetal pig	0.00
"	0.05	Frog	0.00
Rabbit	0.17	Cat	0.00
Chicken	0.02	Human (5 specimens)	0.00
Pig	0.05		

Peptidase.—Peptidase was determined by the usual method of digesting Witte's peptone, glycyltryptophane not being available, and determining the formation of free tryptophane with bromine water. In the tests an equal volume of the skin suspension was mixed with a 10 per cent solution of the peptone and incubated for 72 hours, in some instances for 120 hours. The following table illustrates the relative intensity of the digestive effect of the skin.

Fetal pig	++++	All other human skins	0
Chicken	++	Dog	0
Cat	+	Rabbit	0
Guinea pig	Tr.	Frog	0
Human (normal boy)	"	Pig	0

Lipases.—Porter (3) in his study of the distribution of the lipases of the body has noted the relative richness of the skin tissues in the true lipases and cholesterolases. In view of the function of the skin

in the elaboration of the sebaceous secretion this is to be expected. Porter calls attention to the possible part these ferments play in the relative resistance of the skin to tuberculosis as well as to the bacteriological evidence that the tubercle bacilli in lupus are not only few in number but not infrequently lose their acid-fast character.

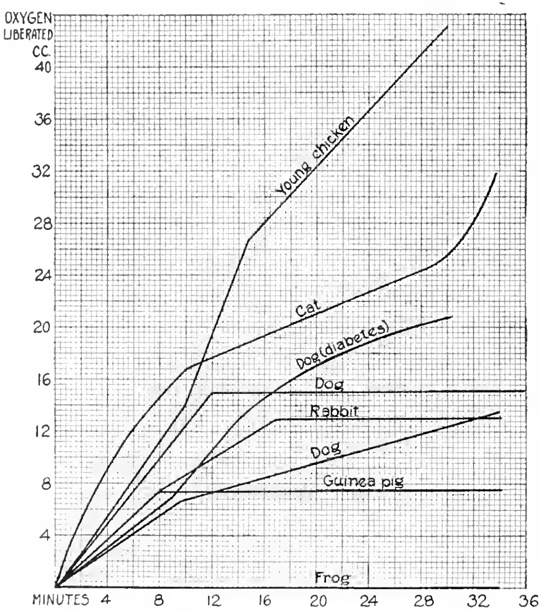
The lipolytic determinations were made with the following substrates: ethyl butyrate, 10 per cent butter fat emulsion, and 10 per cent olive oil emulsion. One experiment was made in which the skin powder was added directly to the olive oil without the addition of water. For purposes of relative orientation we have used a preparation of dog liver, prepared in a similar manner to the skin, with each substrate.

Material.	Substrate.			
	Ethyl butyrate.	Butter fat.	Olive oil emulsion.	Olive oil.
	cc.	*cc.*	*cc.*	*cc.*
Liver	1.5	0.74	3.5	6.0
Pig skin, white	0.5			
" " pigmented	0.37			
Fetal pig skin	0.75			
Human skin	0.1			
" "	0.15	0.6	0.45	0.22
" "	0.1	0.95	0.47	0.14
" "	0.15	0.66	0.3	0.12
" "	0.0			
Chicken "	0.23	2.00	0.5	0.20
Cat	0.23	0.65	0.47	0.15
Frog	0.25	0.59		
Dog	0.25	0.50	0.86	0.15
"	0.20			
"	0.20			
Rabbit	0.20	0.55	0.54	0.35
Guinea pig		0.6	0.75	0.15

In all cases the incubation was for 24 hours at 45°C. and the resulting acidity has been expressed in cc. of 0.05 N sodium hydroxide.

Catalase.—The catalase content of the skin was determined by the ordinary volumetric method and the results are expressed in the accompanying charts in cc. of oxygen liberated by 100 mg. of the dried skin from 50 cc. of a 50 per cent peroxide solution.

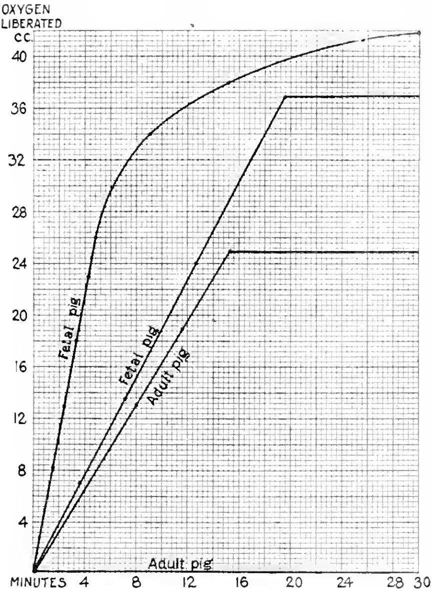
It will be observed that there is considerable variation in the skins, from the 43 cc. liberated in 30 minutes by the young chicken skin to none in the case of frog skin. The skin of the depancreatized dog retained its catalase content despite the fact that the content of the



TEXT-FIG. 2. Determination of the catalase content of the skin of different animals.

other tissues (liver and heart muscle), as determined by Kennedy and Burge (4), is decidedly reduced (Text-fig. 2). A comparison of the fetal and adult skin, made on the skin of pigs, would indicate that the fetal skin contained considerably more catalase than that of the adult (Text-fig. 3).

For purposes of orientation we have studied two normal human skins from accident cases and two skins from pathological postmortem material, one acute alcoholism, the other pregnancy, abortion, and peritonitis. The normal skins are evidently higher in catalase content than the skins from the pathological cases, but without an

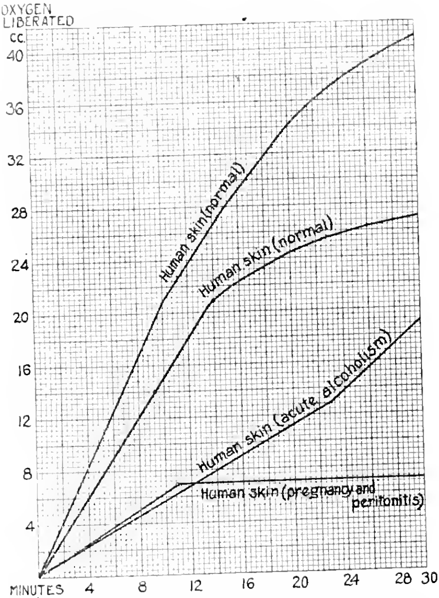


TEXT-FIG. 3. Comparison of the catalase content of the fetal and adult skin.

extended series of observations it would be impossible to draw definite conclusions concerning this point (Text-fig. 4).

Diastatic Ferments.—Progressively diminishing amounts of the skin suspension were incubated for 24 hours with 2 cc. of a 1 per cent solution of starch, and the digestive activity was titrated. The relative diastatic activity of the tissues was found to be as follows:

	$D\frac{37°}{24\ hrs.}$		$D\frac{37°}{24\ hrs.}$
Fetal pig	32	Cat	12
Chicken	16	Human	4
Liver	16	Guinea pig	4
Boy	12	Pig	4
Frog	12	Rabbit	2



TEXT-FIG. 4. Catalase content of two normal skins and two skins from pathological postmortem material, one acute alcoholism, the other pregnancy, abortion, and peritonitis.

DISCUSSION AND SUMMARY.

That a definite change in the reactivity of the skin takes place during the transition from infancy to childhood has been repeatedly noted. Rolly (5), working with a variety of bacterial toxins, found that infants did not react, but that reactions occurred with advancing

age. Similarly Tezner (6), using Witte's peptone, colon bacilli, and tuberculin observed that at about the period that the skin of children became increasingly sensitive to tuberculin, a corresponding reactivity was manifest toward the other antigens. It seems possible to us that this phenomenon is related to the alteration in the ferments of the skin that takes place during the transition period. Thus while the protease of the adult type of skin causes a definite autolysis, that of the fetal skin does not, the synthetic potential evidently predominating. Of greater significance seems to be the fact that the skin of the young animal contains peptidase to a considerable amount, while the adult skin seems to be without this ferment activity.

If we assume that the disintegration of the native protein of the antigen is essential in eliciting certain skin tests, it would seem probable that the splitting would be less apt to take place in the young skin in which the lytic effects of the ferments are relatively suppressed. On the other hand, the young skin, containing peptidase, would be able to digest rapidly any antigen consisting of partially hydrolyzed proteins—as tuberculin—and in this way detoxicate and remove the noxious material. From these considerations the undoubted alteration of the skin reactivity taking place after infancy seems definitely correlated with changes in the proteolytic ferments. In how far they enter into and alter the specific reactions cannot be determined from the limited data so far available.

Of the other ferments, the lipases, as pointed out by Porter, are possibly of importance in the resistance of the skin to tuberculous infection. It will be observed from the table that the activity of these skin ferments is less manifest in their action as esterases, on ethyl butyrate, than on the neutral fats used, oleic oil and butter fat, in this way differing from the activity of the serum. The relative resistance of the skin to tuberculous infection can, however, hardly be due to the activity of the lipase alone, for it must be kept in mind that the lepra bacillus, also rich in lipoids, is enabled to proliferate well within the epithelial tissues.

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INTRAVENOUS SEROTHERAPY OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).*

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(Received for publication, September 4, 1917.)

We have already reported[1] the results of our studies with subcutaneous injections of immune serum in spirochætosis icterohæmorrhagica. In the present article are presented the results of injections made by the intravenous method, which has in the main been employed in the serotherapy of Weil's disease since May, 1916. The number of patients treated in this manner in our clinic from May, 1916, to November, 1916, was 41. We found that the intravenous injection of immune serum far exceeds in potency the subcutaneous injection. An explanation of this finding may be sought in the fact that the therapeutic effect of serum injected directly into the blood stream is 500 times more potent than when it enters the organism by the subcutaneous route (Berghaus).

Experiments to show the comparative effects of the subcutaneous and intravenous administration of serum in Weil's disease were undertaken with seven rabbits, the animals receiving 0.5 to 1 cc. of immune serum per kilo of body weight. At various intervals the blood of the animals was examined for spirocheticidal and spirochetolytic immune bodies. The blood was taken from the ear vein on the side opposite the one in which the intravenous injection was made. The results of these experiments are given in Table I.

The immune bodies could be demonstrated as complete 5 minutes after the intravenous injection of 0.5 cc. of serum per kilo of body weight, while with the subcutaneous method the immune bodies

* Presented at the meeting of the Japanese Hygiene Association, April, 1917.

[1] Inada, R., Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1916, xxiv, 485.

could be detected only 8 hours after the injection and were then not completely absorbed. This passive immunity continued for 3 or 4 days. Complete immunity could not be obtained in man with the use of the small dose injected subcutaneously, but when larger doses

TABLE I.

Immune Bodies in Rabbits Infected with the Blood of Patients Having Weil's Disease.

Animal No.	Method of injection.	Amount injected.	Amount of serum per kg.	Body weight.	Pfeiffer's phenomenon. Before serum injection.	After serum injection. Min. 5	Min. 15	Min. 30	Hrs. 1	Hrs. 3	Hrs. 5	Hrs. 8	Hrs. 12	Hrs. 24	Days. 2	Days. 3	Days. 4	Days. 5	Days. 6	Days. 7	Days. 8
		cc.	*cc.*	*gm.*																	
1	Intravenous.	1 25	0 5	2,500	−		+	+	+							+	+		−		
2	"	2 5	1 0	2,500	−	+		+	+							+	+		−		−
3	Subcutaneous.	2 1	1.0	2,100	−	−		−	−					+	+	+	±		−		
4	"	1.7	0.5	3,400	−			−	−	−	−	−	±	±			±	−			
5	"	1.55	0.5	3,100	−				−	−	−	±	±	±	±	±	±	−			
6	"	3 1	1 0	3,100	−				−	−	−	−	±	±	±	±	±	−			
7	Intravenous.	1.45	0 5	2,900	−	+			+							+	−	−			

Titer of Serum Employed in the Experiments.

Pfeiffer's phenomenon. Amount of serum.	No. of spirochetes.	Course of disease.	Remarks.
cc.			
0.001	Liver emulsion (10 spirochetes per field) 1 cc.	Died 5th day of icterus.	Control died 4th day of icterus.
0.005	" " "	" 16th day without icterus. Autopsy negative.	" " "

− indicates no immune bodies; + immune bodies complete; ± immune bodies incomplete.

were given a complete passive immunity was attained also with the subcutaneous method.

The sera used were obtained from horse's blood as follows: No. 2, February 26, 1916; No. 6, August 7; No. 7, August 7; and No. 8, August 19. One of the horses was immunized from the beginning

with a living culture of *Spirochæta icterohæmorrhagiæ;* four others received first an inoculation of the killed culture, followed later by the living culture. The latter procedure can be more readily carried out. The animal receiving the living culture showed on the 7th day a temperature of 39°C. All the horses were immunized by a uniform procedure.

Titer.—The titer of the immune serum was determined by testing

TABLE II.

Titer of Serum.

No. of serum.	Pfeiffer's phenomenon.		Course of disease.	Remarks.
	Amount of serum.	No. of spirochetes.		
	cc.			
2	0.005	Liver emulsion (10 per field) 1 cc.	Died 12th day of icterus.	Control died 4th day of icterus.
	0.01	" " "	Died 18th day without icterus. Autopsy negative.	" " "
6	0.01	Liver emulsion (10 per field) 1 cc.	Well for more than a month.	Control died 4th day of icterus.
7	0.01	Liver emulsion (10 per field) 1 cc.	Died 10th day of icterus.	Control died 4th day of icterus.
	0.03	" " "	Well for more than a month.	" " "
8	0.01	Liver emulsion (10 per field) 1 cc.	Well for more than a month.	Control died 4th day of icterus.

the quantity which would protect a guinea pig weighing 200 gm. against infection from 1 cc. of pure culture containing 10 spirochetes per field (Leitz $\frac{1}{12}$ oil immersion, ocular 3). The spirocheticidal titer of the serum was 0.01 and 0.03 cc.; it has been found that a titer of 0.01 cc. suffices for the serotherapy of Weil's disease. Table II shows the spirocheticidal and spirochetolytic effects of the immune sera.

The experiments were made with sterile sera without preservative (phenol). As shown in Table II, Sera 2, 6, and 8 were more effective than No. 7.

Dose.—As a rule, 60 cc. of serum were injected intravenously, irrespective of the severity of illness, sometimes the entire quantity being given within 24 hours; or 40 cc. in a day, and the remaining 20 cc. the following day; or 20 cc. daily for 3 successive days. We cannot at present state definitely which procedure is to be preferred, and in order to determine this point, it will be necessary to make observations on a larger number of patients than is here presented. In any case, it is clear that the dose depends upon the severity of the disease. The entire amount of 60 cc. is not needed in the treatment of the less severe cases, although this quantity is always put up for an individual dose. In milder cases, the injection of from 20 to 40 cc. of serum is sufficient.

As is the usual custom, the serum was introduced into the vein of the arm. Previous to the injection 2 to 3 cc. of serum were injected subcutaneously into the chest or the thigh of the patient. The arm injection was then made 2 or 3 hours later, the serum being permitted to run in slowly, allowing 5 minutes for the introduction of 20 cc. The serum should be warmed to body temperature before use.

Day of Injection.—This has an important bearing on the course of the disease. As already emphasized, the action of the immune serum is spirocheticidal and spirochetolytic. The best results are obtained when the injection is made at an early stage of the disease. The question now arises: Up to what day of illness may the injections be continued with success? This period can be determined by studying (1) the infectivity of the patient's blood for guinea pigs, (2) the distribution of the spirochetes in the organs at various stages, and (3) from clinical observations of patients receiving the intravenous treatment. Table III gives the infectivity of the blood on various days of the disease. It is based on 42 cases treated recently, and 69 older cases. It will be observed that the infectivity diminishes gradually in the course of the disease. On the basis of these findings it would seem that the intravenous injection is effective up to the 5th day.

The investigations of Kaneko and Okuda concerning the distribution of spirochetes in the organs in Weil's disease indicated that the liver harbors few or wholly degenerated forms on the 7th day. In two patients on whom a post-

mortem examination was made on the 6th day, spirochetes were found in moderate numbers, though not so numerously as in the guinea pig. According to this, as already stated, we may expect the intravenous injection to be successful up to the 5th day. From our clinical observations, an undoubted effect can be obtained up to the 6th day of illness. We had few cases of this kind, however.

Four patients received the intravenous injection on the 3rd day, 9 on the 4th, 6 on the 5th, and 5 on the 6th day. Patients admitted at a later stage received an initial intravenous injection, not followed by others. 12 of the patients showed no icterus on admission, although 4 had a slightly yellowish pigmentation of the conjunctivæ. Of these 12 patients, 6 failed to develop icterus in the further course of the disease. Classified according to the severity of illness, we treated 6 slightly ill, including atypical cases, 9 moderately ill, and 26 severe cases. At the time that the intravenous treatment was being administered, we had in the clinic 11 other patients who re-

TABLE III.

Infection Experiments on Guinea Pigs with the Blood of Patients Having Weil's Disease.

Day of illness.	Cases previously examined.	Positive cases.	Negative cases.	Per cent positive.	Recently examined cases.	Positive cases.	Negative cases.	Per cent positive.	Total no. of cases.	Positive cases.	Negative cases.	Per cent positive.
2	4	4	0	100.0	0	0	0	0	4	4	0	100.0
3	10	10	0	100.0	4	4	0	100.0	14	14	0	100.0
4	13	13	0	100.0	9	9	0	100.0	22	22	0	100.0
5	12	11	1	91.7	6	5	1	83.3	18	16	2	88.9
6	14	12	2	85.7	5	2	3	40.0	19	14	5	73.7
7	8	4	4	50.0	7	3	4	42.9	15	7	8	46.7
8	4	0	4	0	3	1	2	33.3	7	1	6	14.3
9	1	1	0	100.0	5	0	5	0	6	1	5	16.7
10	0				1	0	1	0	1	0	1	0
11	0				1	0	1	0	1	0	1	0
12	1	0	1	0	1	0	1	0	2	0	2	0
18	1	0	1	0					1	0	1	0
19	1	0	1	0					1	0	1	0
Total No. of cases...	69				42				111			

ceived no serum treatment; *i.e.*, 4 atypical cases, 1 slightly ill, and 6 moderately ill. There were no severe cases among them. It will be noted that the serum was administered in the main only to those who were severely or moderately ill.

Age.—We treated 9 patients between the ages of 16 and 20 years, 9 between 21 and 30, 6 between 31 and 40, 10 between 41 and 50, 4 between 51 and 60, and 3 who were over 60 years of age. We desire to add here that our patients, in addition to the serotherapy, received the benefit of the other usual methods of treatment in Weil's disease; *i.e.*, the intravenous injection of Ringer's solution and the subcutaneous administration of camphor and cocaine.

Spirocheticidal and Spirochetolytic Effect of the Immune Serum Injected Intravenously.

The spirochetolytic and spirocheticidal effects of the serum can be most readily shown when the spirochetes are demonstrable in the peripheral blood by dark-field illumination. In that case no infection experiments need be made. Up to the present time, however, we have observed this to be true in only 2 out of 100 cases. These 2 cases are discussed below.

Case 1.—M., male; age 47 years. The patient was admitted to the clinic on November 4, 1916, on the 3rd day of illness. This was a severe case. The blood showed by dark-field illumination 1 spirochete in from 70 to 140 fields. 1 hour after the intravenous injection of Serum 6, no spirochetes could be detected in two preparations. Blood was then withdrawn from the arm vein, and two guinea pigs received each an intraperitoneal injection of 1 cc. of the patient's blood. The animals became ill and showed typical symptoms 1 or 2 days later than the control animals, which had received blood withdrawn prior to the injection of serum. Although no spirochetes were found in two preparations, the blood was still infectious.

Case 5.—N., male; age 65 years. He became suddenly ill on May 1, 1916, and was admitted to the clinic on May 4, the 4th day of illness. We were able to demonstrate numerous *Spirochæta icterohæmorrhagiæ* in the blood by dark-field illumination in 14 to 16 specimens of 65 to 70 fields each. As a rule, spirochetes are not readily found in fresh preparations. The findings of spirochetes before and after serum injections were as follows:

2 p.m. Spirochetes in the blood, 14 to 16 in 65 to 70 fields.
3 p.m. 17 cc. of Serum 2 injected subcutaneously.
6 p.m. 14 to 16 spirochetes in 65 to 70 fields.

7 p.m. 10 to 16 spirochetes in 65 to 70 fields.

8 p.m. 20 cc. of Serum 2 injected intravenously.

10 p.m. No spirochetes in 2 preparations.

12 m. No spirochetes in 3 preparations.

1 a.m. 2 cc. of patient's blood injected intraperitoneally into a guinea pig, which remained well (May 14).

1.30 a.m. No spirochetes in 1 preparation.

8.30 a.m. 3 cc. of patient's blood injected intraperitoneally into a guinea pig, which remained well.

2 hours after the intravenous injection of serum in Case 5, we were unable to find any microorganisms. This demonstration of the spirocheticidal and spirochetolytic action of the immune serum in man exactly parallels the results obtained with guinea pigs. The patient died, however, at 5.30 p.m. on the following day. But the unexpected results obtained in this case induced us in later cases to employ only the intravenous injection method.

The experiments demonstrate clearly the spirocheticidal and spirochetolytic action of the immune serum, which is capable of destroying the spirochetes in the blood stream within a short period of time.

In the other thirty-nine cases spirochetes could not be readily demonstrated in the blood by dark-field illumination. Hence we conducted infection experiments before and after serum injection, similar to those made with the subcutaneous serum cases (Table IV). Table V gives the results of the infection experiments, the serum administered, and the day of illness when the blood was taken. We have omitted from the table the cases which were negative with the use of blood drawn before serum was injected.

The infection experiments were all negative following the injection of 20 cc. of Serum 2. With Serum 6, only one experiment was positive. In this instance the animal was inoculated 1 hour after the injection of the patient. It appears that Sera 2 and 6 possessed markedly potent spirocheticidal and spirochetolytic properties, while No. 7 was less effective; the titer of the latter serum was 0.03 cc. Although the infection experiments were positive with blood taken after the injection of Serum 7, the animals became typically ill much later than the animals receiving blood drawn before the injection. The difference in the duration of the life of the animals in the two groups was from 3 to 7 days, as in Cases 10, 11, 12, 13, 19, and 25.

TABLE IV.

Infection Experiments with Patients' Blood Withdrawn before and after Intravenous Injection of Serum.

Case No.	Sex.	Day of admission.	Day of illness just before serum injection.	Results on guinea pigs. Before serum injection. No.	Before serum injection. Result.	After serum injection. No.	After serum injection. Result.	Amount of serum injected.	No. of serum.	Time between serum injection and withdrawal of blood.	Day of illness after serum injection.
		1916						*cc.*		*hrs.*	
1	Male.	Nov. 4	3	2	+(7 days). +(8 ").	1	+(9 days).	20	6	1	3
						1	+(9 days).	20		2	3
2	Male.	Sept. 8	3	2	+	2	–	20	7	3	3
3	Male.	Sept. 15	3	2	+	2	–	20	7	3	3
4	Male.	Sept. 22	3	2	+(9 days).	2	+(9 days).	20	7	5	3
5	Male.	May 4	4	1	+	1	–	40	2	5	4
6	Female.	May 30	4	1	+	1	–	20	2	3	4
7	Female.	June 20	4	1	+	1	–	20	2	10	5
8	Male.	Aug. 31	4	2	+	2	–	20	2	3	4
9	Male.	Sept. 22	4	2	+	2	–	20	6	3	4
10	Male.	Sept. 8	4	2	1+(6 days). 1+(8 ").	2	+(13 days).	20	7	3	4
11	Female.	Sept. 19	4	2	1+(6 days). 1+(8 ").	2	+(11 days).	20	7	3	4
						2	–	20		18	5

12	Male.	Sept. 10	4	2,	+(7 days).	2	+(12 days).	20	7	3	4
13	Male.	Nov. 19	4	1	+(6 days).	1	+(11 days).	20	8	3	4
14	Male.	Aug. 28	5	2	1+ 1−	2	−	20	2	3	5
15	Male.	Oct. 20	5	2	−	2	−	20	6	3	5
16	Male.	Nov. 12	5	1	+	1	−	20	6	3	5
17	Male.	Nov. 20	5	1	+	1	−	20	6	3	5
18	Male.	Nov. 26	5	1	+	1	−	20	6	3	5
19	Male.	Sept. 6	5	2	+(7 days).	2	1+(12 days). 1+(13 ").	20	7	3	5
20	Female.	May 28	6	1	−	1	−	20	2	4	6
21	Female.	June 26	6	1	−	1	−	20	2	3	6
22	Female.	Aug. 23	6	1	?	1	?	20	2	3	6
						1	−	40		9	7
23	Male.	Oct. 16	6	2	1− 1?	2	−	20	6	3	6
24	Female.	Oct. 26	6	2	−	2	−	20	6	3	6
25	Male.	Sept. 10	6	2	+(8 days).	2	+(13 days).	20	7	3	6

TABLE IV—*Concluded.*

Case No.	Sex.	Day of admission.	Day of illness just before serum injection.	Results on guinea pigs.				Amount of serum injected.	No. of serum.	Time between serum injection and withdrawal of blood.	Day of illness after serum injection.
				Before serum injection.		After serum injection.					
				No.	Result.	No.	Result.				
		1916						*cc.*		*hrs.*	
26	Female.	Nov. 19	6	1	+	1	–	20	8	3	6
27	Male.	July 25	7	1	+	1	–	60	2	11	8
28	Female.	June 24	7	1	+	1	–	20	2	3	7
29	Male.	July 6	7	1	–	1	–	20	2	3	7
30	Female.	Aug. 31	7	2	–	2	–	20	2	3	7
31	Male.	Sept. 2	7	1	–	2	–	20	2	3	7
32	Male.	Oct. 12	7	2	1+ 1–	2	–	20	6	3	7
33	Male.	Sept. 15	7	1	–	1	–	20	7	3	7
34	Male.	May 20	8	1	–	1	–	20	2	8	8
35	Female.	Nov. 15	8	1	–	1	–	20	7	3	8
36	Female.	Nov. 22	10	1	–	1	–	20	2	3	10
37	Male.	Sept. 15	11	2	–	2	–	20	6	3	11

Cases 1 and 4 are exceptions. It is evident that the serum was potent after 3 hours, although it was incapable in this short period of time of destroying completely the spirochetes contained in the blood. This is true also of Serum 8.

The time elapsing between the injection of serum and the withdrawal of blood varied from 1 to 18 hours, the usual interval being 3 hours. Table VI gives the infection experiments carried out with

TABLE V.

Infection Experiments with Patients' Blood Withdrawn before and after Intravenous Injection of 20 Cc. of Serum.

Positive Infections.

No of serum.	Day of illness.									
	3		4		5		6		7	
	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.
2			4	0	1	0	1	0	2	0
6	1	1	1	0	3	0	1	0	1	0
7	3	1	3	3	1	1	1	1		
8			1	1			1	0		

TABLE VI.

Infection Experiments with Blood Withdrawn after Serum Injections.

Time between serum injection and withdrawal of blood.	Infection experiments before serum injection.	Infection experiments after serum injection.
hrs.		
1–3	+ 19	− 13
		+ 6
Over 5	+ 5	− 4
		+ 1

blood withdrawn after serum injection. These results show that serum having a titer of 0.01 cc., given intravenously in doses of 20 cc., after 3 hours destroys completely spirochetes contained in the peripheral blood, which then is incapable of infecting guinea pigs. While with the use of sera having a titer of 0.03 cc., we found 3 hours after injection incomplete destruction of spirochetes in the blood,

the animals became typically ill later than the controls. It would appear, then, that the spirochetes had become reduced in number or had lost some of their virulence.

Behavior of the Immune Bodies in the Blood.

Experiments were conducted with the blood of twenty patients. Owing to a shortage of guinea pigs, we were not able to inject the animals immediately after withdrawal of the blood, and the fluid was placed in the refrigerator until it could be used. Unfortunately, most of these specimens became contaminated. The results of our investigation for the presence of immune bodies in the blood during the course of the disease, after intravenous injection of immune sera, are shown in Table VII.

The immune bodies appear in the blood much earlier with intravenous than with subcutaneous injections. They are present as

TABLE VII.

Appearance of Immune Bodies in the Serum of Intravenously Injected Patients.

Case No.	Day of illness when serum was injected.	Day of illness when blood was withdrawn.						
		6	7	8	9	10	11	12
8	4	−						+
4	3		−		+			
2	3		−					
11	4	−		+				
10	4			+				
7	4			+				
6	4	±(+7)		+				
14	5				+			
38	5		+					
19	5		+					
15	5		±(+4)					+
23	6	−		+				
24	6	+		+				
21	6				+			
20	6			+				
30	7		−		+			
33	7						+	
28	7				+			
29	7				+			
34	8				+			

early as the 8th or 9th day, which does not, as a rule, occur when the patient has received no serum treatment. They may be observed after the introduction of 60 cc. of immune serum.

In one of the cases referred to the immune bodies appeared as early as the 6th day, before the administration of serum was begun. It is possible that in this instance an error has been made in computing the day of illness, inasmuch as we have never observed a similar result with other non-serum-treated cases.

Mortality.

The total mortality with the use of the intravenous injections was 23.7 per cent. Of forty-one patients under observation, twelve died. We have not included in our tabulations a patient who after recovery from the disease died on the 55th day from complications, a patient admitted to the clinic in moribund condition who died after 29 hours (numerous spirochetes could be demonstrated in the blood), and another, admitted when moribund, who died after 31 hours. The mortality of patients receiving no serum was 30.6 per cent. In the subcutaneously injected patients, the percentage was 17.3. This low mortality in the case of the subcutaneously injected patients is attributable to the fact that in this group, which entered before the 8th day of illness, only five patients were over 40 years of age, while in the group of intravenously injected patients, twelve were over that age.

Since in the moderate and slight forms of Weil's disease death results from complications, we shall consider particularly the severer cases of the disease. As the serum is effective only when administered in the initial stage, we have not considered in our study patients admitted after the 6th day. The mortality of patients who were admitted before the 7th day and received no serum was 57.1 per cent, of the subcutaneously injected patients 40 per cent, and of the intravenously injected patients 38.5 per cent.

It is clear that the total mortality figures, particularly the mortality of the severer cases, is considerably lower in the case of intravenous and subcutaneous serum administration than in the non-serum-treated cases.

In judging the results, one should take into account factors which strongly influence the prognosis. Among these factors must be counted the age of the patient, the severity of the illness, the season of its occurrence, and above all the day of illness on which serum was first administered. One must also consider whether the cases occur in epidemic form or sporadically. The difficulty of estimating the results is further increased by the fact that often at the onset it is not possible to judge precisely the degree of severity of the disease, while on the 6th or 8th day an error in prognosis is unusual. Table VIII shows the influence of the age of the patient upon the prognosis.

TABLE VIII.

Mortality and Age.

Age.	Without serum treatment.		Intravenous serum treatment.
	Inada.	Nagao.	
yrs.	*per cent*	*per cent*	*per cent*
Up to 20	12.5	60.0	0
21–30	21.0	17.9	12.5
31–40	23.5	20.0	0
41–50	21.4	35.0	42.8
51–60	75.0	50.0	33.3
Over 60	50 0	75.0	100.0

Of 21 patients less than 40 years old, admitted from the 3rd to the 7th day of illness, who were treated by intravenous injections of serum, only 1 died. This was a patient admitted on the 7th day. The 21 cases included 4 slightly ill, 7 moderately ill, and 10 severely ill patients. Of the 12 patients over 40 years of age—2 slightly ill and 10 severely ill—7 died. Prior to the age of 40, a good result is insured from the serum injections, but after 50 the outlook is not favorable; the older persons constitute, in fact, the severest cases of the disease. When we compare 10 severe cases below 40 years with 10 above that age, we find among the first only 1 death as against 7 deaths in the older group. It is evident that the years above 40 exert an unfavorable influence on the prognosis of Weil's disease, and this is especially true when the disease appears in severe form.

Effect of Intravenous Serotherapy on the Spirochetes in the Organism.

The spirochetolytic action of the immune serum upon the spirochetes in the human body is more pronounced with the intravenous than with the subcutaneous injection. Kaneko and Okuda have studied this phase of the subject.[2]

Effect of Serotherapy upon the Symptoms and Course of Weil's Disease.

Severity of Illness.—When the serum is administered early, the disease appears to assume a milder form. As already stated, it is difficult at the beginning to judge the precise degree of illness. We observed one patient moderately ill and another slightly ill, who after the serum injection could be grouped with the atypical cases. It is, however, not possible to assert that this change is wholly attributable to the serum; it may be that the disease in these cases would have run a less severe course without the administration of serum. But when we study the influence of the intravenous injection upon the different symptoms, we find that on the whole they are considerably diminished and shortened in duration.

Fever appears not to be influenced by the serum to any extent, even in cases where serum is administered early in the disease. In fact we often observed a rise in temperature after an injection, though it is not possible to say that this was due to the action of the serum.

The immune serum has a favorable effect on the duration of the icterus. The exact time of disappearance cannot be definitely stated on the basis of an examination made of the skin and mucous membranes, inasmuch as anemia usually follows recovery. We have, therefore, taken as an index Gmelin's urine test, which has been used in our clinic during the past 10 years. Without serum treatment, the icteric condition usually continues for a period of 20 days or more in the severe cases, sometimes as long as 30 days; and in the moderately ill 14 to 30 days, occasionally more than that time. In the cases receiving subcutaneous serum treatment, the icterus continued for an equal length of time. No definitely favorable effect was observed, even in the cases receiving an injection as early as the 3rd or 4th

[2] Their work is reported in detail on page 305 in this *Journal*.

day of illness. On the other hand, the icterus of the intravenously treated patients in most cases disappeared within 20 days, in the earliest case on the 13th day. No such finding was ever observed in the non-serum-treated cases or in those receiving subcutaneous injections. Of the severe cases receiving the intravenous injection prior to the 6th day, in only two did the icterus last more than 20 days. The longest period was 12 to 14 days in moderately severe cases. When the injection of serum is deferred until after the 6th or 7th day, the icteric condition will continue as long as it would if no serum had been given. We cannot state definitely whether an injection made on the 1st or 2nd day is capable of suppressing the icterus completely, but this is probable. Of the eight patients who received serum treatment on the 3rd or 4th day and at that time showed no signs of icterus, three remained without jaundice throughout. In any event we may say that the duration of the icterus is shortened considerably when the intravenous injections are given early in Weil's disease. The intensity of the icterus in most cases is greatly diminished by the intravenous injections (Tables IX, X, and XI).

The serum has likewise a decided influence upon hemorrhages. This was observed to a slight extent also with the subcutaneous injections. As a rule, cutaneous hemorrhages continue, but they are less pronounced in character. The percentage of petechiæ in the patients receiving intravenous treatment was 70.7, while in those receiving no serum it was 72.2. Although there is little difference in the figures, the duration of this symptom in the first group was considerably shorter. In half of the non-serum-treated cases the petechiæ continued for 10 days, while in the cases which received serum intravenously (admitted from the 1st to the 6th day of illness) only a third showed this symptom for more than 9 days. The number and size of the petechial spots were also reduced. Hemorrhage from the nose occurred also with serum treatment, but the percentage was somewhat smaller; that is, 29.3 against 32.7 per cent. Hemorrhages from the mucous membranes, gums, and tongue, and hematoma of the buccal mucosa continued to occur. In the intravenously treated cases the proportion was 41.5 per cent, in the non-serum-treated cases, 43.5 per cent. When the intravenous injection

was given early, within the first 6 days, the percentage was 30.6. This is true of intestinal hemorrhages, which may be reduced one-half by the intravenous injections. Table XII gives in percentages the

TABLE IX.

Duration of Icterus with Intravenous Serum Injection.

Day of illness when serum was injected.	Degree of illness.	Duration of icterus.
		days
3 (four cases).	Atypical.	Without icterus. Icterus only of conjunctiva bulbi.
	"	
	Severe.	13
	"	17
4 (five cases).	Atypical.	Without icterus.
	Severe.	15
	Moderately severe.	14
	Severe.	15
	"	23
5 (five cases).	Moderately severe.	Without icterus.
	Severe.	8
	Moderately severe.	12
	Severe.	16
	"	21
6 (six cases).	Atypical.	Without icterus. Icterus only of conjunctiva bulbi.
	Slight.	
	Moderately severe.	11
	Slight.	12
	Moderately severe.	13
	Severe.	18
7 (six cases).	Severe.	16
	Moderately severe.	14
	" "	16
	Severe.	17
	"	23
	"	30
8 (two cases).	Moderately severe.	16
	Severe.	28
9 (two cases).	Moderately severe.	13
	Severe.	23
Total 30		

hemorrhages occurring in the non-serum-treated cases, the intravenously treated cases, and of the latter those receiving serum injections at an early stage. The table also shows the percentages of hematemesis and epistaxis.

TABLE X.

Duration of Icterus with Subcutaneous Serum Injection.

Day of illness when serum was injected.	Degree of illness.	Duration of icterus.
		days
3 (one case).	Atypical.	4
4 (one case).	Severe.	22
5 (three cases).	Severe. Moderately severe. Severe.	16 20 24
6 (six cases).	Slight. Severe. Moderately severe (approaching slight form). Severe. Moderately severe. Severe.	14 18 20 20 29 33
7 (three cases).	Severe. " "	20 24 30
8 (five cases).	Slight. Moderately severe (approaching slight form). Moderately severe. " " " " (approaching severe form).	10 20 24 25 26
9 (one case).	Severe.	20
13 (one case).	Severe.	17 (died on 34th day).
Total 21		

From the above considerations we may conclude that the early intravenous injection of immune serum has a decidedly favorable influence upon the hemorrhagic tendency of Weil's disease, and particularly upon hemorrhages from the mucous membranes.

TABLE XI.

Duration of Icterus without Serum Treatment in Thirty-Six Cases.

Degree of illness.	Duration of icterus.
	days
Atypical.	Icterus only of conjunctiva bulbi.
"	5
Slight.	11
"	12
Moderately severe.	14
" "	14
" "	17
Slight.	17
Moderately severe.	18 (died on 18th day).
Severe.	20
"	20
Moderately severe.	21
Slight.	22
Moderately severe.	22
Severe.	24
"	24
"	24
"	25
"	25
Moderately severe.	22
" "	25
" " (approaching severe form).	25
" "	26
" "	28 (had beri-beri).
" "	29
" "	30
" " (approaching severe form).	30
Severe.	30
"	31
"	34
"	37+
Moderately severe.	37+
" "	38+
" "	24+
Severe.	24+
"	29+ (died on 38th day).

TABLE XII.

Percentage of Hemorrhages in the Serum-Treated and Non-Serum-Treated Cases.

Symptoms.	Without serum treatment.	Intravenous serum treatment.	Intravenous serum treatment (26 cases admitted from .–6 days).
	per cent	*per cent*	*per cent*
Hemorrhages from tongue and mouth	43.5	41.5	30.6
Intestinal hemorrhages	30.2	22.0	15.3
Hematemesis	11.3	9.7	7.6
Bloody stools	15.1	7.3	7.6
Petechiæ	72.2	70.7	69.2
Epistaxis	32.7	29.3	
Complications—suppurative processes	24.0	14.6	4.9

We have not observed a decidedly favorable influence of the serum upon the pulse, although arrhythmia was a less frequent symptom. It occurred in 20 per cent of the cases, while in the non-serum-treated cases the figure was 50 per cent.

Complications such as suppurative processes—parotitis, skin abscesses—were found in 14.6 per cent, in patients admitted from the 3rd to the 6th day only in 4.9 per cent, and in patients receiving no serum treatment in 24 per cent of the cases. Thus these complications are greatly reduced with the early use of the serum.

Summarizing the observations on such symptoms as hemorrhage, particularly of the mucous membranes, heart rhythm, and suppurative processes, which play a large part in the outcome of the disease, we are justified in saying that the intravenous injection of immune serum has a definitely beneficial effect.

The after-fever was found to occur somewhat more frequently—in 34.1 per cent—while without serum treatment it occurred in only 28.2 per cent. The greater frequency of this symptom is probably referable to the fact that the most severely ill patients who recover from the disease are included in this category. As the time of greatest mortality of Weil's disease lies between the 8th and the 18th days, and the after-fever, as a rule, does not begin until the 13th or 15th day, it is evident that this symptom does not usually occur in the cases ending fatally. The percentage of after-fever is found to increase as the mortality decreases.

On two occasions following the injection of Serum 7, and once after Serum 2, the patients had chills followed by a rise in temperature. Serum rash occurred three times after the administration of Serum 2. There were no anaphylactic manifestations.

Among 18 patients who returned for examination after several months we found 8 who showed more or less marked ocular disturbances. These 18 cases comprised 10 severe, 5 moderately severe, and 3 atypical forms of the disease. The 8 patients showing ocular disturbances were as follows: 5 from the severely ill group, 1 from the moderately ill, and 2 from the atypical group. 1 patient showed, on examination in the ophthalmologic clinic, iridocyclitis, hypopyon of the right eye, and opacity of the vitreous humor; in another only the last symptom was present. These after-effects seem to occur more frequently in the severe types of the disease. We cannot say at this time to what extent the sequelæ are influenced by serum treatment.

DISTRIBUTION OF SPIROCHÆTA ICTEROHÆMORRHAGIÆ IN THE ORGANS AFTER INTRAVENOUS SERUM TREATMENT.

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(Received for publication, September 4, 1917.)

The material here reported was obtained from eight cases of Weil's disease in which postmortem examinations were made. The patients had all received intravenous serum treatment before the 8th day of illness. The distribution of spirochetes within the organs differs somewhat in these patients from that in those receiving no serum treatment. The latter have already been described by us.[1] In Table I are given our findings on the density of the spirochetes in the various organs, and also the findings in four non-serum-treated cases showing no complications.

It will be observed that in the serum-treated cases the spirochetes are more sparsely distributed than in the other group. In the former we were able to demonstrate microorganisms constantly in the kidneys, often in the heart muscle and the appendix, but only once in the liver, suprarenals, and lymph glands, and in three cases in muscle tissue. The spirochetes were few in number and the specimens observed were markedly degenerated. With the exception of one case, but few spirochetes could be discovered in the liver, while in the non-serum-treated cases in this stage of the disease there was no difficulty in demonstrating spirochetes in that organ, although they were not numerous. In a patient dying on the 6th day, who had received no serum treatment, we found a dense distribution of spirochetes in the liver. The pancreas, with the exception of one case, and the spleen, lungs, and other organs of the serum-treated patients showed no spirochetes.

[1] Kaneko, R., and Okuda, K., *J. Exp. Med.*, 1917, xxvi, 325.

TABLE I.

Spirochetes Found in the Organs in Weil's Disease.

Serum-Treated Cases.

Case No.	Age.	Sex.	Day of illness when serum was given.	Amount of serum.	Day of death.	Liver.		Kidneys.		Supra-renals.	Spleen.	Lymph glands.	Appendix and intestine.	Pancreas.	Lungs.	Heart.	Bladder.	Prostate.	Muscle.
						Extra-cellular.	Intra-cellular.	Inter-stices.	Tu-bules.										
	yrs.			*cc.*															
1	30	Male.	5	20	5	−	−	+	−	−	−	−	+	−	−	+		−	−
2	23	"	5	20	5	−	−	**+**	+	−	−	−	+	+	−	−	**+**	+	+
3	57	"	4	20	6	−	−	**+**	+	−	−		+	−		**+**	**+**	−	+
			5	20															
4	56	Female.	6	20	8	−	−	+	**+**	+	−	−	−	−	−	+	−		−
			7	20															
5	50	Male.	5	20	9	−	−	+	+	−	−	+	−	−	−	+		−	−
			6	20															
			7	20															
6	30	"	7	20	11	−	−	+	+		−	−	−	−	−	−			+
			8–10	40															
7	47	"	3	20	11	−	−	−	+	−	−	−	+	−	−	−		−	−
			4	20															
			5–6	20															
8	73	Female.	4	20	11	−	+	**+**	+		−	−	+		−	−			−
			5	20															

Non-Serum-Treated Cases.

9	59	Male.			6	**+++**	**+**	**++**	+	**++**	+			**+**	+				
10	45	"			7	+	+	**++**	+		+	+	**+**	**++**	+	**++**	**+**	**++**	+
11	28	"			8	−	+	**+++**	**+**	+	+	+	**+++**	+	−	**++**	**++**	**++**	+
12	14	Female.			10	+	+	**+++**	**+**	+	+	+	+	+	+	**+**	+		**+**

+, very few spirochetes—one or a few in a preparation.

+, spirochetes sparsely distributed. The spirochetes can be found readily, more than 10 to 20 in a preparation, or one in several fields.

++, relatively numerous spirochetes, one or a few in a single or several fields, with numerous spirochetes in a preparation

+++, numerous spirochetes, some being found in almost every field, and often numerous specimens in a single field.

−, negative finding in one or more preparations.

CONCLUSIONS.

On the basis of these findings we believe that we are justified in saying that the immune serum of Weil's disease is capable of destroying the spirochetes found within the organs in man, with the exception of the kidneys, and that the action of the serum upon the spirochetes is spirochetolytic and spirocheticidal. The scattered spirochetes in the kidney, on the other hand, are resistant to the action of the immune serum.

The spirochetes disappear almost completely from the organs during the convalescent stage of Weil's disease, even when no serum has been administered. The only organ to be excepted is again the kidney, but no comparison between serum-treated and non-serum-treated cases should be made in this respect, for spirochetes are found numerously in the kidneys even with serum treatment.

The disappearance of the spirochetes from the organs and tissues in Weil's disease seems to be not so marked with the subcutaneous serum treatment as with the intravenous method, but the manner of their disappearance is about the same.

We desire to express to Professor R. Inada, our appreciation of his interest in the work.

THERAPEUTIC EXPERIMENTS WITH ROSENOW'S ANTI-POLIOMYELITIC SERUM.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATES 8 TO 10.

(Received for publication, January 2, 1918.)

Investigators are divided into two well defined groups with reference to the interpretation of the relation of certain streptococci cultivated from poliomyelitic nervous tissues to epidemic poliomyelitis. One group[1] affirms that the streptococci bear a causal relation to poliomyelitis and are even related biologically to the globoid bodies of Flexner and Noguchi,[2] while another group[3] denies that they possess any essential etiologic importance and views them merely as secondary invaders.[4]

The question at issue is an important one in every way, because upon its true answer will depend the prophylactic measures adopted to prevent epidemics of poliomyelitis and the direction which effort will take in perfecting an efficient agent for specific therapy.

Until recently the effort made to treat cases of poliomyelitis specifically has been with the blood serum of convalescent and recovered cases of the disease. This procedure is based upon several kinds of conclusive experimental data. However, too few observations are available to decide whether the method gives unmistakable thera-

[1] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

[2] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[3] Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557. Kolmer, J. A., Brown, C. P., and Freese, A. M., *J. Exp. Med.*, 1917, xxv, 789.

[4] Smillie, W. G., *J. Exp. Med.*, 1918, xxvii (in press).

peutic results in human cases of poliomyelitis, although the indications are favorable.[5]

The employment of the serum derived from recovered cases of poliomyelitis followed not only on account of the detection of its neutralizing property *in vitro* for the poliomyelitic virus, but also because of the failure to induce antibody formation in a variety of domestic animals, including the horse, by the injection of the nervous tissues of monkey or man carrying the virus.[6] Incidentally, it may be stated that only imperfect success in developing antibodies in rabbits and monkeys has attended the repeated injection of cultures of the globoid bodies.[7]

A far greater measure of success has been claimed for the streptococci in producing antibodies for the virus of poliomyelitis. Rosenow[8] and Nuzum and Willy[9] assert that animals immunized with the streptococci cultivated from poliomyelitic cases exhibit various antagonisms to the virus. Monkeys inoculated with streptococci are said to be protected from subsequent infection with the poliomyelitic virus; the blood of the protected monkeys is stated to be neutralizing *in vitro* for the virus; and finally, horses immunized with the virus are said to yield a serum which possesses neutralizing, protective, and therapeutic properties, even when applied to man.

It is this last statement which calls for painstaking control. It is obvious that to obtain a decision from the treatment of human cases of poliomyelitis would require a large and varied series of observations extending over a long period of time and embracing epidemics of considerable magnitude and various degrees of severity. Moreover, the observations would have to be carefully controlled by comparison with an equal number of cases, occurring simultaneously, untreated with the antiserum, under approximately identical conditions and equally accurately studied. To secure these data might require several years, as has been the case notably with the serum treatment of diphtheria and epidemic meningitis. The question arises,

[5] Amoss, H. L., and Chesney, A. M., *J. Exp. Med.*, 1917, xxv, 581.

[6] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

[7] Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 545.

[8] Rosenow, E. C., *J. Am. Med. Assn.*, 1917, lxix, 261, 1074.

[9] Nuzum, J. W., and Willy, R. G., *J. Am. Med. Assn.*, 1917, lxix, 1247.

therefore, whether a method is not available by means of which a probable decision may be reached more expeditiously and with greater certainty. It is because of our belief that a decisive experimental method is at hand that the series of experiments to be reported were performed.

EXPERIMENTAL.

The injection intracerebrally into monkeys of minute quantities of an active virus of poliomyelitis is followed by paralysis and, as a rule, by death of the animal. The injection of far greater quantities of the same virus into the blood stream produces no symptoms. If, however, as Flexner and Amoss[10,11] have shown, the meninges and choroid plexus are chemically inflamed by a simultaneous or previous injection of sterile horse serum, monkey serum, or even isotonic saline solution, the virus is enabled to pass from the blood into the nervous tissues and thus to induce the characteristic changes which lead to paralysis and even to death. The same authors found only one substance which, when injected intraspinally, prevented the localization of the virus in the nervous organs after intravenous injection, and that is the serum derived from monkeys which have survived a poliomyelitic infection. Moreover, this serum is capable of setting aside the effects of the chemical inflammation incited by horse serum or other foreign fluids injected intraspinally. In other words, when the immune convalescent serum is introduced into the meninges in animals previously injected intraspinally with horse serum or other fluids mentioned, followed by an intravenous injection of the virus, no paralysis or other evidence of infection results. This experiment gives such decisive and unequivocal results that it seems particularly adapted to determine the therapeutic value of a serum or other product reputed to be effective in the treatment of poliomyelitis in man.

The experiments to be described were carried out in the following manner. An active, fresh poliomyelitic virus was obtained in the usual manner by inoculating a monkey intracerebrally with a suspension of glycerolated virus. On the 1st day of complete prostration, the animal was etherized, and the brain and spinal cord were

[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[11] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

aseptically removed. With the spinal cord and medulla, a 5 per cent suspension in isotonic salt solution was prepared, shaken, and centrifuged, and the clear supernatant fluid injected intravenously into *Macacus rhesus* monkeys. Two separate sets of experiments were performed. Control animals and animals treated with Rosenow's serum, with normal horse serum, and with convalescent monkey serum, in the same manner, were tested simultaneously. The protocols follow. The outcome is as sharp and hence as decisive as the experimental results could make it.

Experiment 1.—Monkey A, control. Nov. 13, 1917. Injected intravenously 50 cc. of the virus prepared as described. The animal remained well.

Monkey B, normal horse serum control. Nov. 12, 1917, 5.10 p.m. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 13, 11.15 a.m. Injected intravenously 50 cc. of virus. 12 noon. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 14. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 15. Repeated intraspinal injection of normal horse serum. Nov. 16. Repeated intraspinal injection of normal horse serum. Protects left leg; ataxic. Nov. 17, a.m. Left arm, right deltoid, and both legs paralyzed; head tremor; ptosis of the left eyelid; almost prostrate. 4.30 p.m. Died.

Autopsy.—Macroscopic and microscopic lesions of poliomyelitis. No visible changes in viscera.

Monkey C, Rosenow's serum. Nov. 12, 1917, 4.40 p.m. Intraspinal injection of 3 cc. of Rosenow's antipoliomyelitic horse serum activated with one-ninth volume of fresh guinea pig serum. Nov. 13, 10.45 a.m. Intravenous injection of 50 cc. of virus. 10.50 a.m. Intraspinal injection of 3 cc. of Rosenow's serum. Nov. 14, 15, 18, 19, and 20. Injected 3 cc. of Rosenow's serum, intraspinally. The clinical course of the animal was as follows: Nov. 17. Slow. Nov. 19. Excited; slow; protects right leg. Nov. 20. Drags right leg; climbs awkwardly. Nov. 22. Both legs weak. Nov. 23. Legs paralyzed; deltoids weak. Nov. 24. Prostrate (Fig. 1). Nov. 26. Moribund; etherized.

Autopsy.—The spinal cord and brain were edematous and the gray matter was congested. Microscopic examination of the central nervous system showed marked perivascular infiltration and some neurophagocytosis in the gray matter of the medulla and cervical enlargement characteristic of poliomyelitis. Perivascular infiltration (Figs. 2 and 3), congestion, neurophagocytosis, and meningeal infiltration in lumbar enlargement. Focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in the posterior root ganglia.

Monkey D, serum of recovered monkeys. Nov. 12, 1917, 4.55 p.m. Intraspinal injection of 3 cc. of mixed serums from several *rhesus* monkeys which had recovered from experimental poliomyelitis and subsequently received subcutaneous injections of the virus contained in the spinal cord and medulla (reinforced

immune). Nov. 13, 11 a.m. Intravenous injection of 50 cc. of virus suspension followed by intraspinal injection of 3 cc. of immune serum. Nov. 14, 15, 18, 19, and 20. Intraspinal injections of 3 cc. of immune serum. The clinical course of this animal was in striking contrast with the preceding. At no time were any symptoms present; the animal continued apparently normal throughout the treatment and is well at the present time (Jan. 1, 1918).

This experiment was repeated with precisely the same results. The protocols follow. In the second experiment, the normal horse serum control was omitted.

Experiment 2.—Monkey E, control. Nov. 26, 1917. Intravenous injection of 50 cc. of the virus. No symptoms appeared, and the animal has remained normal up to the present time (Jan. 1, 1918).

Monkey F, Rosenow's serum. Nov. 26, 1917, 6 p.m. Intraspinal injection of 2 cc. of activated Rosenow's antipoliomyelitic horse serum. Nov. 27, 11.35 a.m. Intravenous injection of 50 cc. of virus, followed immediately by the intraspinal injection of 2.5 cc. of Rosenow's serum. The intraspinal injections were repeated on Nov. 28, 29, Dec. 2, and 3. In each instance the activated serum was injected. Dec. 3. The animal developed a marked tremor of the head, ataxia, and right facial paralysis; also, the deltoid muscles were weak. Dec. 4. The monkey died in the early morning.

Autopsy.—Macroscopic lesions of poliomyelitis throughout brain and cord. Microscopic examination of the central nervous system showed marked congestion and perivascular infiltration, slight cell degeneration, and neurophagocytosis in medulla and cervical enlargement (Fig. 4); slight meningeal infiltration in lumbar enlargement, and focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in posterior root ganglia (Fig. 5).

Monkey G, immune monkey serum. Nov. 26, 1917, 5.15 p.m. 2 cc. of pooled immune serum injected intraspinally. Nov. 27, 12.25 p.m. Intravenous injection of 50 cc. of the virus, followed by the intraspinal injection of 2.5 cc. of pooled immune serum. The intraspinal injections of the pooled serum were repeated on Nov. 28, 29, Dec. 2, and 3. At no time were any symptoms detected, and the animal is normal at this time (Jan. 1, 1918).

DISCUSSION.

The preceding experiments accomplish two purposes directly. First, they test the ability of Rosenow's serum, which was prepared by injecting a horse with cultures of the streptococci derived from poliomyelitic nervous organs, to prevent a poliomyelitic infection arising in the monkey after an intravenous injection of the virus. This is readily accomplished by means of the immune serum obtained

from convalescent and recovered monkeys. Second, they compare directly under these favorable therapeutic conditions the Rosenow serum with the serum of immune monkeys.

The results of the experiments are unequivocal. They show the Rosenow serum to be devoid of protective power. Moreover, they show that the Rosenow serum acts in the manner of normal horse serum in promoting infection in monkeys from an intravenous injection of the virus, in itself incapable of inducing paralysis.

The immune monkey serum possesses, under the same conditions of administration, perfect protective power, as has been shown previously by Flexner and Amoss.[11]

A further conclusion may be drawn from the experiments. Rosenow states that the horse serum prepared by him contains demonstrable antibodies for the streptococci employed in its production. It is assumed that these antibodies are identical with the antibodies, demonstrable by neutralization experiments with the virus, contained within human and monkey serum derived from recovered cases of poliomyelitis in man and the monkey. This supposition is rendered untenable by the results of our experiments. The antibodies induced in the horse by immunization with the streptococci have proven incapable of neutralizing the virus of poliomyelitis introduced into the blood of monkeys in its passage to the central nervous system—a neutralization which the immune monkey serum readily effects. The two classes of antibodies or immunity principles, those present in the blood derived from recovered cases of poliomyelitis and those induced in the horse by treatment with streptococci, are therefore to be regarded as distinct.

There is a further corollary to this general deduction. Once it is established that the antibodies yielded by the streptococci differ essentially from those induced by the virus of poliomyelitis, the contention that virus and streptococci are identical becomes untenable. In other words, the experiments reported in this paper tend also to refute the claim that certain streptococci are the microbic cause of epidemic poliomyelitis.

Rosenow and Nuzum and Willy assert that their serums possess striking therapeutic activity in man. Their conclusions are based on the treatment of relatively small numbers of cases of epidemic polio-

myelitis during the past summer and autumn. We have already drawn attention to the difficulties surrounding a statistical study, of limited extent, of the questions here involved. Hence we venture to place, in this instance, the greater weight on decisive animal experiments, and those reported in this paper clearly show that Rosenow's horse serum injected intraspinally into monkeys is without specific protective power against the virus of poliomyelitis.

It has, however, been shown by Flexner and Amoss[12] that normal horse serum, when injected intraspinally into monkeys, promotes the passage of poliomyelitic immune bodies from the blood into the subarachnoid space. Hence it is possible that under certain circumstances in which those bodies are already present in the blood in man, they may be directed into the subarachnoid space through the increased permeability of the meninges induced by the horse serum and thus affect the course of the infection. The antibodies have been detected on the 3rd[13] and 6th[12] days of illness in man, or, in other words, early in the course of the disease.

This, however, is a purely hypothetical consideration, in support of which normal horse serum should prove as effective as antistreptococcus serum. It is questionable whether this roundabout method of directing the circulating immunity bodies to the central nervous organs is advisable in practice. As far as present knowledge, based on definitive experiments, is concerned, it may be said that only immune serum derived from convalescent and recovered cases of poliomyelitis in man and the monkey have been determined to be protective against the infectious power of the poliomyelitic virus.

CONCLUSIONS.

Two series of experiments are described in which Rosenow's antipoliomyelitic serum, so called, has been compared with the immune serum derived from monkeys which have convalesced or recovered from experimental poliomyelitis.

The experiments consisted in introducing an active virus of polio-

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

[13] Kling, C. A., and Levaditi, C., Études sur la poliomyélite aiguë épidémique, Paris, 1913, p. 114.

myelitis into the blood and of injecting the two kinds of serum into the cerebrospinal meninges according to the method of Flexner and Amoss.

Under the conditions of the experiment, the control monkeys (*a*) receiving the virus intravenously alone do not develop paralysis, while those (*b*) receiving the virus intravenously and normal horse serum intraspinally develop paralysis. Moreover, the monkeys (*c*) receiving the virus intravenously and Rosenow's antipoliomyelitic serum intraspinally develop paralysis in the manner of those receiving normal horse serum intraspinally. The monkeys (*d*) which received the virus intravenously and the convalescent or immune monkey serum intraspinally alone did not develop paralysis.

The Rosenow serum acts in the manner of normal horse serum; it promotes the passage of the virus of poliomyelitis from the blood into the nervous organs, and it does not protect from infection.

We have found no evidence that Rosenow's serum under the conditions of the tests is effective therapeutically in monkeys or possesses antibodies of the same nature as those present in the blood of monkeys which have recovered from experimental poliomyelitis.

Since the antibodies in convalescent poliomyelitic serum in man and the monkey are identical, it follows that any antibodies present in the Rosenow horse serum do not conform to those occurring in human convalescent serum.

EXPLANATION OF PLATES.

The illustrations were taken from monkeys treated with Rosenow's serum.

Plate 8.

Fig. 1. Monkey C. 11 days after the intravenous injection of virus. Received seven intraspinal injections of Rosenow's serum. Arms, legs, and back muscles paralyzed; face muscles active.

Plate 9.

Fig. 2. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in anterior horn. × 165.

Fig. 3. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in posterior horn. × 165.

PLATE 10.

FIG. 4. Monkey F. Cervical enlargement showing anterior horn with degeneration of ganglion cells and neurophagocytosis. × 240.

FIG. 5. Monkey F. Posterior root ganglion with ganglion cell degeneration, neurophagocytosis, and mononuclear cell infiltration. × 240.

Fig. 1.

(Amoss and Eberson. Rosenow's antipoliomyelitic serum.)

FIG. 2.

FIG. 3.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)

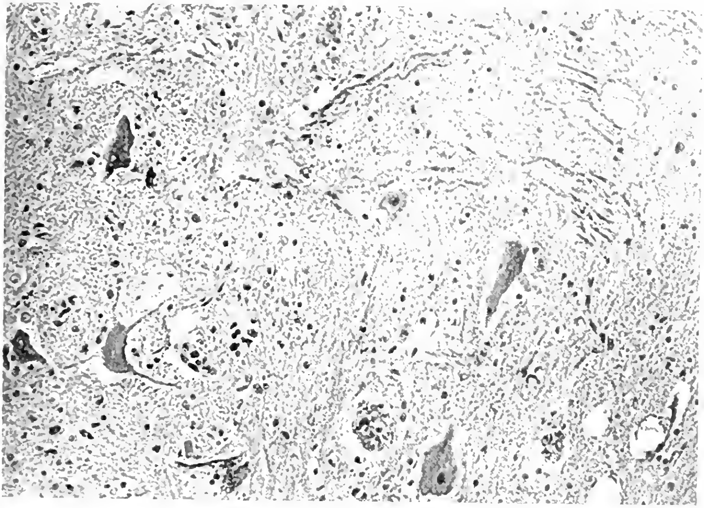

FIG. 4.

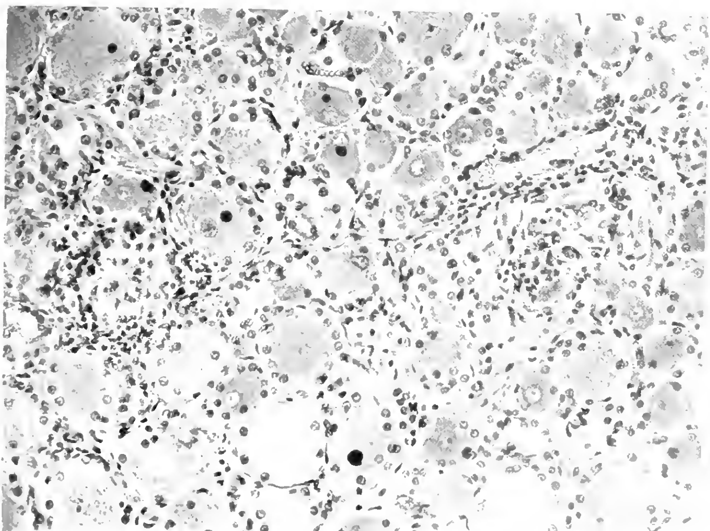

FIG. 5.

CULTIVATION EXPERIMENTS ON THE GLOBOID BODIES OF POLIOMYELITIS.*

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(Received for publication, December 6, 1917.)

The microbic cause of poliomyelitis is a subject still in the foreground of interest with respect to the etiology of that disease. The present paper deals with the conditions surrounding the cultivation of the globoid bodies described by Flexner and Noguchi (1) from poliomyelitic tissues and is intended as a contribution to the technique of the method.

The technique of the cultivation is a complex and difficult procedure, even more difficult, perhaps, than the isolation of *Treponema pallidum*. With respect to the treponema, the experimenter is assisted by the fact that it is comparatively large, possesses clear-cut morphological characteristics, and is motile—properties which make its detection relatively easy.

The globoid bodies, on the other hand, are extremely minute and non-motile. They are not readily distinguished from tiny particles of detritus in the early generations of scantily growing cultures. Hence weeks, even a month or more, may elapse between the initial inoculations of the culture medium and the definite determination of successful cultivation of the organism; and it may happen that the growth then observed may fail to develop on transplantation, so that the opportunity for its identification may be lost. The difficulties surrounding the method of cultivation have resulted in the confirmation of Flexner and Noguchi's work by only a few bacteriologists, the larger part of those who have attempted to repeat it failing in their efforts.

The present study was undertaken in the hope of modifying the

* This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

method by rendering it simpler and more certain of success. The original procedure is not only uncertain in its ultimate results, but also in the number of cultures yielded by a given lot of tubes inoculated with poliomyelitic tissue. Sometimes of 30 to 50 tubes thus inoculated, one or two only would yield cultures, the others remaining without demonstrable growth. Where the conditions all appear to be so similar, it would seem that a higher percentage of cultures should be obtained. Obviously the conditions within the tubes varied in an unaccountable manner; and it became also the purpose of this study to eliminate the factor of variation and thus to increase the proportion of cultures obtainable from a certain number of inoculated tubes.

Hitherto, the globoid bodies have been cultivated from the central nervous organs, which are the seat of the profounder effects of the disease. Now, according to present conceptions of the pathology of poliomyelitis, the disease partakes often of the nature of a general or systemic infection. Indeed, the non-paralytic or abortive cases, so called, pursue a course unattended by paralysis or even by marked symptomatic involvement of the central nervous system. Hence it became desirable to study the general viscera by culture methods.

The source of the tissues submitted to cultivation was *rhesus* or *cynomolgus* monkeys in which the inoculation form of the disease had been produced. The usual procedure was to permit the paralysis to develop to the point at which the inoculated animals became prostrate; they were then etherized. In certain instances animals were used which had been prostrate for several days or which had died some hours earlier. A comparison of the cultures from the two classes of animals—those recently paralyzed and still in good physical condition with those which have been *in extremis* for some time or have actually succumbed to the disease a number of hours before autopsy—is illuminating as regards the number and kind of cultures obtained.

The virus or emulsion of the nervous organs employed for inoculation was either an active virus which has been transmitted in the laboratories of The Rockefeller Institute for several years, or the less active M.A. virus (2), which has also passed through many monkeys. The mode of inoculation was usually intracerebral, but

sometimes it was by other routes—the nose and blood. A brief summary of the clinical data of the infected monkeys is given in Table I.

We propose to give the details of the technique of the successful cultivations of the globoid bodies, which will be followed by a description of the results obtained by the method, including both success and failure, and the probable causes of each.

TABLE I.

Summary of Clinical Data of Monkeys Infected with Poliomyelitic Virus.

Serial No.	Source of virus.	Amount inoculated.	Route of inoculation.	Duration of symptoms	Duration of prostration.	Length of illness	Pathologic appearance.
				days	*days*	*days*	
1	Fresh mixed.	50.0 cc.	Intravenous.*	1½	½	7	Typical.
2	Glycerolated mixed.	2.0cc suspension	Intracerebral.	4	1	13	"
3	Fresh mixed.		Nasal.	9	6	18	"
4	" "	0.2 cc. filtrate.	Intracerebral.	3	1	10	"
5	" "	50.0 cc.	Intravenous.*	2	0	7	"
6	" "	0.1 "	Intracerebral.	1½	¼	10	"
7	" "	0.1 "	"	1	0	11	"
8	" "		Nasal.	3	1	11	"
9	" "	0.1 cc.	Intracerebral.	2	1	7	"
10	" "	0.2 "	"	8	1	13	" †
11	" "	0.2 "	"	7	3	15	" †
12	M.A.	1.0 "	"	5	1½	9	"
13	Fresh mixed.		Nasal.	4	2	12	"
14	" "		"	2	½	10	"
15	" "	2.0 cc.	Intracerebral.	2	1	6	"
16	" "		"	2	½	6	"
17	" "		Nasal.	3	1	7	"
18	M.A. cultivated.	2.0 cc. mass culture.	Intracerebral.	5	0	12	"

* Intravenous inoculation of centrifuged emulsion following the injection of horse serum intraspinally.

† These animals had been dead 7 and 3 hours respectively when autopsied.

Cultivation Technique.

The methods used for the cultivation of the globoid bodies from the tissues of monkeys are essentially the same as those originally

described by Flexner and Noguchi (1). Certain variations of the technique will, however, be described. In part, the technique is a repetition of previously reported work, but is given as a convenient summary.

Test-tubes, 1.5 by 20 cm., should be used for all experiments, for the narrower tubes are much more likely to become contaminated. They should be plugged with cotton and placed in a surgical package containing 25 to 30 tubes, which should then be sterilized and not opened until the set of tubes is to be used.

Fresh sterile tissue is added to each tube. A healthy, fat, adult rabbit is chosen, one that has not even a scratch or other detectable defect upon any part of its body. The animal, which must be given no food for 24 hours, is lightly etherized and bled to death from the heart by means of a sterile needle and a large bulb pipette. All hair is then removed from the abdomen and anterior thoracic walls of the animal's body by sodium sulfide solution, and the skin is prepared with the usual surgical procedures, soap and water, bichloride solution, alcohol, and finally iodine. Instruments for obtaining the kidneys should not be boiled, but required instruments are made into a set and sterilized in a glass container in the hot air sterilizer. One set is used to open the skin, another set to open the peritoneal cavity, and a fresh pair of scissors and forceps are used to remove the kidneys. All procedures should be carried out in a dust-proof room, or under a hood, the walls of which have been wiped down with bichloride solution.

After removal to a large sterile Petri dish, the capsule is stripped from the kidney, each kidney is cut into 20 to 25 pieces, and one bit of tissue placed in each sterile test-tube. This procedure must also be carried out in a dust-proof room. While removing the capsule of the kidney and cutting it into pieces, the assistant should hold the cover of the Petri dish directly over the field of operation in order to avoid dust contamination.

The poliomyelitic material which is added to each tube consists of a bit of tissue of about the same size as the bit of rabbit kidney tissue. The infected monkey should not be allowed to die, nor, in fact, to become prostrate, but should be etherized at the height of the active symptoms. After etherization, the animal should be bled, and the visceral tissues, liver, spleen, kidney, thymus, etc., removed with exactly the same technique as was used in obtaining sterile rabbit kidney. It is important to use a separate pair of forceps and scissors for each organ.

The technique for obtaining uncontaminated poliomyelitic brain tissue is as follows: The calvarium is removed with aseptic precautions, great care being taken not to injure the dura. The dura over the hemisphere opposite the one that had been injected is then seared with a red hot knife blade, and the dura is opened with sterile instruments. A portion of the cerebrum is removed with a separate set of instruments and placed in a sterile Petri dish. This material is at once taken to a dust-proof room, when it may be cut into suitable pieces and placed immediately in the tissue test-tubes.

Methods for removing bits of sterile spinal cord are very unsatisfactory. The following method has proved the most useful. The entire cord is exposed under aseptic precautions, great care being taken not to injure the dura. A portion of the cord, 3 to 4 cm. long, is tightly tied at either end with a sterile thread and the portion removed. This bit of cord with intact dura is placed in bichloride alcohol for 60 seconds, then rapidly passed through five separate washings of sterile salt solution. The dura is wiped dry with sterile gauze, seared with a narrow hot knife blade, and is then opened with sterile instruments and the cord cut into 0.5 cm. segments, each portion being put into a tissue test-tube.

When the test-tubes have been inoculated with rabbit kidney and poliomyelitic material, ascitic fluid should be added, 15 or 18 cc. being sufficient. The ascitic fluid must, of course, have been tested carefully for sterility, both for aerobic and anaerobic organisms. It should have a specific gravity of at least 1,015, be clear, free from bile, not more than 3 months old, and should be stored in the refrigerator. It should not be heated above 50°C. or filtered. Chylous or bloody ascitic fluid is not suitable. *Before adding the ascitic fluid to the inoculated test-tubes, it should be warmed to 45°C. and placed under a vacuum to remove the air.*

Complete anaerobiosis must be obtained if one is to be successful in the cultivation of the globoid bodies. In our first experiments the vacuum jar was used, but the results were disappointing. The vacuum jars were either so poorly ground that they would not hold a vacuum, or, owing to defects in the glass, they cracked or exploded under the tremendous negative pressure. These difficulties were finally met by the use of the hydrogen-nitrogen jar, which utilizes the catalytic action of platinized asbestos upon hydrogen and oxygen, and removes all traces of oxygen from the jar. The details of this apparatus have already been published (3). Subsequent experiments with this jar have given most satisfactory results. The pyrogallic acid-sodium hydroxide indicator has remained unchanged in color for weeks, showing complete anaerobiosis; and the method is so rapid, clean, and simple that it seems to promise a simple satisfactory solution for the problem of anaerobic technique.

The inoculated ascitic fluid-tissue tubes, usually twenty-five to thirty tubes, are placed in the anaerobic jar and incubated for 11 to 12 days. Control tubes, inoculated with brain and other tissues, are incubated in the open air and examined daily in order to determine the presence of contaminating organisms.

After 12 days' incubation, the jar is opened, and 0.2 cc. of the ascitic fluid is removed from the bottom of each tube, without disturb-

ing the bits of brain or kidney tissue. The special pipettes devised by Gates (4) are very useful for all poliomyelitic culture work. The material is transferred directly into freshly prepared tissue tubes. 15 cc. of air-free, warmed ascitic fluid are added to each tube as before, the tubes placed in the anaerobic jar, and at once returned to the incubator. The original tubes may be placed in the refrigerator to be examined at leisure.

The second generation of ascitic fluid-tissue tubes is incubated for 12 days and the anaerobic jar is then opened. As before, immediate transfer is carried out, 0.2 cc. of the ascitic fluid near the bottom of the tube being transferred to freshly prepared tissue tubes. To a second set of tissue tubes is added 0.1 cc. of the material from the bottom of the tubes of the second generation. To Set 1, containing 0.2 cc. of transferred material, is added warmed air-free ascitic fluid as before. To Set 2, containing 0.1 cc. of the material, are added 15 cc. of semisolid medium which has been prepared as follows:

Veal infusion agar, 2 per cent, with a reaction of +0.5 to phenolphthalein, is melted, boiled, and then rapidly cooled to 50°C. Ascitic fluid, clear, straw-colored, free from bile, with a specific gravity of 1,015 or higher, is warmed to 45°C. and placed under a vacuum to remove the air. 1 part of the melted agar, cooled to 50°C., is added to 2 parts of warmed ascitic fluid, and 15 cc. of the mixture are added to each inoculated test-tube of Set 2.

The two sets of tubes are at once placed in separate anaerobic jars and incubated. The second generation of ascitic fluid tubes from which the transfer was made is placed in the refrigerator and examined on subsequent days for the presence of the globoid bodies.

The anaerobic jar containing the set of semisolid tubes (Lot 2) is incubated for 8 days, and the jar is then opened and the tubes are examined. If any of the tubes contain suspected colonies in the depths of the semisolid medium, these tiny colonies are removed by means of a capillary pipette and examined microscopically. If positive, fresh semisolid tissue tubes are made from each colony, and these tubes are placed in the anaerobic jar and incubated. If none of the tubes show visible colonies, they are all replaced in the anaerobic jar and reincubated for 8 days. If at the end of this reincubation there is no growth in the semisolid tubes, the set is discarded.

The anaerobic jar containing the ascitic fluid tubes of the third

generation, Lot 1, is incubated for 12 days, then opened, and fresh sets of ascitic fluid and semisolid tissue tubes are made exactly as described for the second generation. This process of making ascitic fluid and semisolid tubes is continued for at least five generations, after which time, if no growth has been obtained, all tubes are discarded. Every original tube inoculated is therefore a separate experiment and must be given its own number, and a detailed record must be kept of each subsequent generation of that culture. Each lot of ascitic fluid tubes, after their period of incubation, is kept in the refrigerator, so that one may subsequently return to the earlier generation in case it is necessary to pick up a positive culture that may have died out in subsequent generations.

For example, Culture 6, brain tube No. 1, after growing for two generations in semisolid medium, refused to grow further in this medium, so that it was necessary to return to the ascitic fluid tube, brain tube No. 1, third generation, from which the strain had originally been obtained, and though the growth was very scanty, and the tube had been in the refrigerator for more than a month, nevertheless a positive permanent culture was obtained.

Criteria for the Determination of the Globoid Bodies.

It is necessary to have definite criteria for the determination of the globoid bodies. These have been described in the original publication by Flexner and Noguchi (1) and in later publications and need simply be summarized for convenience.

Morphology and Staining Characteristics.—Morphology and staining characteristics are somewhat variable factors, the variation depending not so much upon the organism as upon the observer, and, though confirmatory, they are insufficient indices in the identification of an organism.

Characteristic Growth in Semisolid Medium.—It is not enough to determine the presence of an organism in the ascitic fluid medium which seems to the observer to possess the characteristic morphology of the globoid bodies. Semisolid medium should be inoculated and observed daily. There should be no apparent growth in the medium for 3 or 4 days. At the end of 72 hours or later, the characteristic minute colonies begin to appear, being definitely established only at the end of 6 to 7 days. In the anaerobic jar, the line of demarcation of growth is about 1 cc. from the surface of the medium. If the semisolid tube has been incubated in the open air, however, only a few colonies will be seen about the tissue in the bottom of the tube and extending upward about 1 cc.

Action upon Carbohydrates.—An additional factor for the identification of the globoid bodies depends upon their inability to attack carbohydrates. Tissue tubes of ascitic dextrose broth are inoculated with a small fragment of a semisolid culture. The broth should be sugar-free veal infusion, +0.2 to +0.3 acid to phenolphthalein. To each 5 cc. tube of broth is added an equal amount of unheated sterile ascitic fluid plus sufficient 10 per cent sterile sugar solution to give a concentration of 0.5 per cent. The hydrogen ion concentration of the medium should be approximately that of blood neutrality; namely, 7.4. Control tubes should be made both of uninoculated tubes and of tubes inoculated with various types of streptococci. The tubes should be incubated in the anaerobic jar for 7 days and the hydrogen ion concentration determined. The globoid bodies will not attack the simple sugar, whereas the control tubes of streptococci will show a marked increase in acidity.

Results Obtained in the Cultivation of the Globoid Bodies.

Material from eighteen poliomyelitic monkeys has been used in the attempted cultivation of the globoid bodies. For the first four experiments, the anaerobic technique used was the vacuum jar, and in each instance the experiment was unsuccessful. For all the later experiments, the platinized asbestos method was used, and far more satisfactory results were obtained.

The material from three of the monkeys was contaminated with a pure culture of streptococcus in the first generation in many or all of the tubes. This contamination was not due to faulty technique, because all control tubes were negative, but was the result of a secondary invading streptococcus which is commonly found in the blood and tissues of animals and human beings who have been prostrate and moribund for hours or days before death finally occurred.

Of the remaining eleven monkeys, the typical globoid bodies were cultivated from seven, and a total of twenty-two separate cultures or strains was obtained. The largest number of strains obtained from a single monkey was six, whereas in two instances only one strain was obtained. Besides the twenty-two strains, eleven other strains were cultivated in the ascitic fluid medium, but I was unable to transfer them to semisolid medium, and they are, therefore, not included in the total number of cultivations. The eleven strains, incompletely isolated, were derived in part from monkeys yielding completely isolated strains. Table II summarizes the successful and partially successful results of the cultivations.

TABLE II.

Successful and Partially Successful Cultivation of the Globoid Bodies.

Serial No.	Generation carried before discarding.	Virus cultivations in semisolid medium.			Virus cultivations in ascitic fluid medium but not transferred to semisolid medium.		
		No. of strains obtained.	Source.	Generation in which culture was obtained.	No. of strains obtained.	Source.	Generation in which culture was obtained.
1	3rd	None.			None.		
2	4th	"			"		
3	12th	"			1	Mesenteric node.	4th
4	9th	"			1	Kidney.	4th
5	10th	1	Brain.	3rd	1	Brain.	4th
6	12th	2	" 1, cord 1.	5th 6th	1	"	4th
7	10th	4	Brain.	4th	None.		
8	11th	3	"	4th	"		
9	3rd	None.			"		
10	2nd*	"			"		
11	1st*	"			"		
12	12th	5	Brain.	1, 3rd 1. 4th	"		
13	8th	6	" 4, spleen 2.	4th	1	Adrenal.	4th
14	7th	None.			3	Brain 2, spleen 1.	3rd
15	4th	"			1	Brain.	3rd
16	4th	"			2	"	3rd
17	1st*	"			None.		
18	4th	1	Brain.	3rd	"		
Total........		22			11		

* All tubes contaminated with a streptococcus.

Nineteen of the completely isolated strains were obtained from brain substance, one strain was cultivated from the cervical portion of the spinal cord, and two were cultivated from the spleen. Of the eleven incompletely isolated strains, seven were cultivated from the brain, one from the kidney, one from a mesenteric node, and one from the spleen. The shortest period of time between the making of the cultures at autopsy of the monkey and definite establishment of a positive culture was 28 days, and in the third generation. The longest

period required for the definite establishment of a strain in semisolid medium was 54 days, although the organism in this case had been found in the ascitic fluid tube in the third generation on the 30th day.

In no instance was a definitely positive culture found in the first generation of tubes, but usually the organisms were present in sufficient numbers in the second generation to suggest at least that the result would eventually be positive. The globoid bodies are so small and may so readily be confused with detritus, that even a presumptive decision should not be reached unless characteristic forms are found under at least five different microscopic fields. The organisms are so few in number in the second generation that a presumptive test, though possible, is a long and tedious process. It is much simpler and more satisfactory to make subcultures of all tubes of the second generation into the semisolid medium and thus reach a positive conclusion in the succeeding generation by means of the typical growth in colony form.

Occurrence of Streptococci and Other Organisms.

The occurrence of streptococci in certain series of the cultures has been noted. More rarely other microorganisms than streptococci were cultivated instead of or with the globoid bodies. We should consider the sources and endeavor to estimate the significance of these classes of organisms. It is obvious that culture tubes which require so much manipulation will sometimes become contaminated. This condition will account for the miscellaneous bacteria sometimes encountered in the tubes, but probably not for the streptococci which have come to occupy a position of prominence, even if not of importance, with regard to the vexed question of the etiology of poliomyelitis.

The streptococci, when present in the cultures, have not, as a rule certainly, entered with the ascitic fluid or the kidney fragment or from the air. There is no reason to doubt that they have been introduced with the fragment of nervous or other tissue with which cultivation was attempted. Whether they are also to be regarded as contaminations in the broad sense is the question at issue.

A summary of all the extraneous microorganisms encountered (Table III) reveals that a large proportion was streptococci. It is of interest to have found that the tubes prepared from the liver and

kidney of the monkeys were more frequently attended by growth of streptococci, and other extraneous organisms, than those of the spleen, and the tubes of the spleen more often showed extraneous organisms than those of the brain.

In three monkeys there was a rich growth of a pure culture of streptococci in all the tubes of the first generation, the control tubes remaining sterile. In all these instances the animal had been prostrate 24 hours or longer, and in two instances had been dead several hours when the autopsy was performed. This finding is in such definite contrast with the results of the cultures prepared from the corresponding tissues of monkeys severely paralyzed but not yet prostrate and moribund or dead that it would seem to throw considerable

TABLE III.

Summary of Contaminations with Streptococci and Other Organisms in Poliomyelitic Cultivation Experiments.

Tissue.	No. of original culture tubes made.	No. of tubes contaminated with streptococci in 1st generation.	No. of tubes contaminated with streptococci in all later generations.	No. of tubes contaminated with organisms other than streptococci in all generations.
Brain	137	25	11	10
Spinal cord	14	0	7	0
Kidney	31	7	5	0
Liver	31	5	2	3
Spleen	30	6	1	2
Adrenal	17	3	1	1
Mesenteric node	19	2	0	0
Thymus	10	2	0	0
Pancreas	4	0	0	0
Total	293	50*	27†	16

* Forty-six of the fifty tubes that were contaminated with streptococci in the first generation were obtained from four monkeys that had been prostrate and moribund for a considerable time before autopsy. The remaining four streptococcic contaminations in the first generation of tubes were from fourteen other monkeys, autopsied under more favorable conditions.

† An average of more than five subcultures from each original tube was made so that the total number of contaminations with streptococci in all subsequent generations (27) represents the extraneous contaminations in over 1,000 tissue culture tubes.

light on the nature of the streptococcal invasion, detected by Mathers (5), Rosenow, Towne, and Wheeler (6), and Nuzum and Herzog (7) in human beings and monkeys who have succumbed to poliomyelitic infection.

In order to obtain more light on this subject, we cultivated, by means of the technique employed for the globoid bodies, the tissues of monkeys which had succumbed in the laboratory to tuberculosis, dysentery, and other diseases, and isolated, in several instances, streptococci from the liver, kidney, spleen, and nervous tissues.

The streptococci yielded by the tissues of the moribund and dead poliomyelitic monkeys were transplanted into carbohydrate media and injected into rabbits. The fermentation reactions were those of streptococci in general and sharply distinguished them from the globoid bodies. The inoculations gave results so closely in accordance with those described by Bull (8) as not to call for restatement here.

Typical Cultivation Experiments.

Experiment A.—February 23. *Macacus cynomolgus.* Inoculated into left cerebral hemisphere with 0.1 cc. of an N Berkefeld filtrate of a centrifuged 5 per cent suspension of glycerolated brain and spinal cord carrying mixed virus. March 4. Tremor of head, slow movements, weakness of arms. March 5. Extensive paralysis; animal unable to rise. Etherized and bled to death from the heart. The autopsy showed the presence of typical lesions of poliomyelitis.

Cultures were made as follows: brain, 8 tubes; spinal cord, 4 tubes; intervertebral ganglion, 1 tube; kidney, 3 tubes; liver, spleen, thymus, 2 tubes each. Rabbit kidney fragments from a single animal were employed, but two specimens of ascitic fluid were used. The even-numbered tubes received ascitic fluid, Lot 7, the odd-numbered tubes Lot 10. The control tubes consisted of ascitic fluid plus kidney, ascitic fluid alone, and a kidney tissue-ascitic fluid inoculated with stock culture of globoid bodies No. 973. All the tubes were placed in the hydrogen-nitrogen jar and incubated, and in addition three tubes of ascitic fluid plus kidney and brain were incubated in the open air and examined every other day. The latter series remained sterile and was discarded on the 8th day.

March 15. Hydrogen jar opened. Sodium pyrogallate solution was colorless; hence the jar had been oxygen-free. Fresh kidney tissue-ascitic tubes were reinoculated from each of the original tubes except the thymus tube. The fragment of thymus had floated to the top, and the tube was discarded. Rabbit kidney No. 9 was used for the new tubes; and for the even-numbered tubes ascitic fluid No. 7 and in the odd-numbered tubes ascitic fluid No. 6 were used. The usual controls were added and all placed as before in the anaerobic jar.

March 17. The microscopic examination of the first generation tubes was negative throughout.

March 26. The second anaerobic jar had remained oxygen-free. In removing the tubes, spleen tube No. 20 and brain tubes Nos. 7 and 8 were broken. Microscopic examination of the remaining intact tubes gave the following result:

Brain Tube No. 1.—Organisms suggestive of globoid bodies.

Spinal Cord Tubes Nos. 10 and 12, Ganglion Tube No. 13, and Spleen Tube No. 19.—Indefinite bodies, somewhat suggestive of the globoid bodies.

Cord Tube No. 11 and Liver Tube No. 17.—Grossly contaminated.

March 26. Ascitic fluid-kidney tissue tubes were inoculated from the second generation, using ascitic fluid No. 13 for the odd-numbered and No. 6 for the even-numbered tubes. Anaerobic cultivation.

April 4. The jar had remained free of oxygen. Microscopic examination of the tubes gave the following result:

Brain Tube No. 1.—No growth.

Brain Tube No. 5.—Same as No. 1.

Other tubes of the third generation suggestive of globoid bodies were: brain No. 6, cord No. 12, and kidney No. 16.

April 4. Tubes of semisolid medium were inoculated and placed in the anaerobic jar.

April 10. Jar opened. All the cultures were negative except brain No. 6, cord No. 12, intervertebral ganglion No. 13, and spleen No. 19, which were merely suggestive, and cord No. 10, which contained a growth of the globoid bodies. Transplantation from the tubes which were suggestive and the one positive to fresh tubes of semisolid medium gave no result. Thus far the cultivation experiments with this specimen of virus, which had been carried through 7 or 8 weeks, resulted in three cultures of the globoid bodies which could not be developed in the fourth generation.

The ascitic fluid-kidney tissue culture of March 26 was preserved in the refrigerator for 20 days. Transplantation from it was again made into fluid and semisolid media. Brain tube No. 1 gave a positive growth which again failed to grow in the next transplantation. But by returning again to the fluid culture of March 26, a strain of brain No. 1 culture was secured which continued to grow in semisolid medium. Similarly the ascitic fluid-tissue tube of cord No. 12, made on April 14 from the mother tube of March 26, was positive and yielded a culture capable of growing in subcultures.

Thus from a total of more than 100 tubes carried over a period of more than 2 months and through five or six generations, two strains of the globoid bodies which bore subculturing were finally obtained. In each instance the organism was detected in the second generation; but patience and persistence were needed to obtain strains which would continue to grow in artificial media. All the cultivation ex-

periments were not so long, tedious, and difficult as this one; but it has been given in detail to illustrate the intricacy of the problem of the cultivation of the globoid bodies.

Experiment B.—April 15. *Macacus rhesus.* A cotton plug, containing 1 gm. of fresh mixed virus, was placed in the left nares, where it was allowed to remain for 16 hours. April 22. Right arm was paralyzed. April 25. Paralysis has slowly progressed; animal was almost prostrate but was bright. April 27. Extensive paralysis; complete prostration. Etherized and bled from the heart. Autopsy showed the presence of typical lesions of poliomyelitis in the cord and brain.

Cultures were made as follows: Brain, 8 tubes; kidney, 2 tubes; spleen, 3 tubes; adrenal, 2 tubes; liver, 3 tubes; mesenteric nodes, 2 tubes; and pancreas, 2 tubes; a total of 22 tubes. Three lots of ascitic fluid were used and kidney tissue from two rabbits, the same ascitic fluid being placed in every third tube and the same rabbit kidney tissue in every alternate tube. Controls were made as usual. The hydrogen-nitrogen jar was used and was satisfactory throughout the experiment.

May 8. The anaerobic jar was opened and all tubes were found to be in good condition except one liver tube, which was broken. A complete set of tissue tubes was made up, one rabbit kidney and two fresh ascitic fluids being used. These were placed in the hydrogen-nitrogen jar as before. Microscopic examination of the tubes of the first generation showed no growth in any tube.

May 20. The anaerobic jar of May 8 was opened. Examination of the tubes revealed a typical microscopic picture of the globoid bodies in brain tubes Nos. 1 and 5. One spleen tube and one liver tube were contaminated with a large Gram-positive bacillus and were discarded. Semisolid tissue culture tubes were made from each of the remaining tissue culture tubes of the second generation and placed in a hydrogen-nitrogen jar.

May 29. The anaerobic jar of May 20 was opened. A few tiny colonies were to be seen in the bottom of the following tubes: brain No. 4, brain No. 5, brain No. 6, spleen No. 12, spleen No. 13, and adrenal No. 11. Microscopic examination confirmed the presence of the globoid bodies. There was no growth in the other tubes, and they were discarded.

Fresh semisolid tissue tubes were at once made of all the suggestive semisolid cultures of the third generation and also from the ascitic fluid tubes of the second generation, which had been kept in the ice box since May 20. These tubes were all placed in the anaerobic jar as usual.

The fourth generation tubes resulted in the definite establishment of four brain strains, three from the semisolid media of May 29 and one from the ascitic fluid media of May 20. The two spleen strains were also definitely established from the spleen semisolid tubes of May 29. Adrenal tube No. 11 yielded a typical growth in the third generation, but it refused to grow in all subsequent generations and therefore cannot be included in the series.

In this experiment, therefore, a total of six positive cultures was obtained, four from the brain and two from the spleen, out of a total of twenty-two original tubes. There were two contaminations in the series, both occurring in the second generation.

Carbohydrate Reactions of the Globoid Bodies.

In their original publication Flexner and Noguchi (1) state that the globoid bodies have no ability to split the polysaccharides, alcohols, or even the simple hexoses. Since it is obvious that if an organ-

TABLE IV.

Reaction of the Globoid Bodies upon Simple Sugars.

Strains.	Strain No.	Dextrose.		Lactose.	
		Titration to phenol-phthalein in the cold.	pH	Titration to phenol-phthalein in the cold.	pH
New strains.	5	0.60	7.3	0.72	7.3
	6 (Brain No. 1).	0.56	7.3	0.60	7.2
	6 (Cord " 12).	0.62	7.3	0.62	7.3
	7	0.52	7.25	0.50	7.3
	8	0.50	7.3	0.64	7.2
	12	0.64	7.2	0.68	7 2
	4 (Brain No. 3).	0.46	7.3	0.64	7.2
	4 (Spleen " 13).	0.56	7.3	0.58	7.3
Stock strains.	1281	0.56	7.2	0.48	7.3
	1328	0.52	7.2	0.60	7.2
	973	0.56	7.3	0.70	7.3
Controls. Streptococci.*	1623–2	6.6	5.3	5.6	5.6
	1556	4.56	5.8	3.1	6.0
Controls.					
Kidney + ascitic broth.		0.60	7.1	0.62	7.2
Ascitic broth alone.		0.54	7.25	0.54	7.3

Titration results are expressed in the number of cc. of 0.1 N sodium hydroxide which would be required to neutralize 100 cc. of the medium.

The hydrogen ion concentrations were done by the Henderson-Palmer colorimetric method (9).

* Both strains of streptococci were obtained from the first generation of brain tissue tubes from poliomyelitic monkeys.

ism is unable to affect simple sugars, it will also be unable to affect higher ones, the first necessary experiment is to determine the reaction of the organism upon simple sugars.

In our experiments dextrose and lactose only were used. The medium employed is described in the paragraphs upon technique. There are so many buffer salts in an ascitic bouillon medium that the determination of acid production by titration with 0.05 N sodium hydroxide is not satisfactory. Instead, one should use one of the various methods for the determination of the hydrogen ion concentration.

Eleven strains of the globoid bodies were inoculated into the sugar media. Eight of them had been isolated within the past 3 months, and three were stock cultures that had been cultivated in the laboratory for one or more years. Controls were added to the series of two strains of streptococci which, as contaminations, had been isolated from poliomyelitic monkeys during the previous 3 months. Controls also were added of tubes containing ascitic broth plus rabbit kidney tissue and ascitic broth alone. The tubes were incubated in an anaerobic jar for 7 days, at the end of which time there was a good growth in each tube. The results of the experiment are summarized in Table IV. Thus none of the globoid bodies were able to attack the simple sugars, while the two strains of streptococci attacked them vigorously.

Inoculation of Monkeys with Cultures of the Globoid Bodies.

That the inoculation of pure cultures of the globoid bodies, even in a remote generation, will sometimes produce infection in monkeys, attended by the symptoms and specific lesions of experimental poliomyelitis, has been shown by the reports of Flexner and Noguchi (1), and Flexner, Noguchi, and Amoss (10). On the other hand, their experiments indicate that it is exceptional for the cultivated globoid bodies to exhibit definite pathogenic properties. However, the observation was made that in some animals in which a single inoculation failed to cause any symptoms, a subsequent one was followed by typical paralysis attended by the specific lesions in the central nervous organs of the infected monkeys.

The question naturally arose whether any of the cultures of the globoid bodies isolated by me possessed pathogenic properties. Eight different strains were inoculated into *Macacus rhesus* monkeys. In some instances a single injection, in others several injections were given. Three of the inoculated animals developed some degree of paralysis following intracranial or intraspinal inoculation. In one instance the culture was in the fourth, in the remaining two in the fifth generation. The other five animals did not develop suspicious symptoms. A brief recapitulation of the protocols of the three monkeys showing paralysis is given, followed by a discussion of the significance of the experiments.

Experiment C.—Macacus rhesus. May 19. Left intracerebral inoculation of 2 cc. of an ascitic fluid culture M.A. in the fifth generation of the globoid bodies derived from the brain of a monkey. May 25. Animal had a convulsion, following which it was ataxic and dazed. Lumbar puncture yielded a turbid fluid under pressure containing 1,400 cells, chiefly lymphocytes, and + + globulin with 0.1 cc. May 26. Left arm protected; slight left facial palsy; ataxia. May 28. Facial paralysis marked. Following lumbar puncture brief convulsion. Cerebrospinal fluid clearing; excess of lymphocytes. May 31. Condition improved; probably will recover; etherized for cultures and histology. The autopsy revealed a cyst at the point of inoculation, containing yellow fluid. A filtrate of the local site and an emulsion of the spinal cord and medulla were inoculated into two *rhesus* monkeys, respectively; neither developed symptoms.

Cultures were prepared from the brain, kidney, liver, spleen, mesenteric node, and adrenals. All tubes were carried through three generations without contamination. The globoid bodies were isolated from one tube only, brain tube No. 2.

Sections were prepared from the medulla, cervical and lumbar regions of the spinal cord, and intervertebral ganglia. In none were any lesions characteristic of poliomyelitis found.

The globoid bodies employed for inoculation were derived from the M.A. virus, which, in its present condition, is of low virulence for monkeys. The inoculation gave rise to a local cyst, which rarely results from the intracerebral inoculations, attended by a distinct left facial palsy and weakness (?) of the left arm. It is doubtful whether the symptoms denoted experimental poliomyelitis. Probably the facial palsy was related to the cyst formation at the inoculation site; as definite paralysis did not appear in the arm, the appearance of

weakness may have been deceptive. The complete absence of histological lesions is inconsistent with the production of typical experimental poliomyelitis; although since the only developed paralysis was facial, it is always possible that exhaustive microscopic study might have revealed poliomyelitic lesions in the nucleus of the facial nerve. However, the failure of the filtrate and tissue emulsion to transmit the disease to other monkeys also speaks against the condition having been experimental poliomyelitis.

On the other hand, the injection of the globoid bodies gave rise to a marked cellular reaction in the cerebrospinal fluid, in which the predominating cells were lymphocytic. Ordinary bacteria which set up a meningitis usually produce polymorphonuclear cells. But the chief point of importance is the great difficulty and slight success attending the recovery, by cultivation, of the globoid bodies, even from the inoculated nervous tissues. Once these cultivated microorganisms readapt themselves to the condition of growth within the living body, they resist artificial cultivation as do the original tissue parasites—a point noted in Flexner and Noguchi's first communication.

Experiment D.—Macacus rhesus. June 9. Inoculated into the left cerebral hemisphere with 2 cc. of a lightly centrifuged unwashed sediment of a 5 day old mass culture of the globoid bodies isolated from the spleen of a monkey. The strain of the globoid bodies had passed through five generations and been under cultivation for 55 days. This animal developed convulsions, ataxia, and weakness of arms and legs. A lumbar puncture performed on June 14 yielded fluid not under pressure, containing ++ globulin 0.1 cc. and 110 mononuclear cells per c. mm. June 24. The symptoms had all diminished. The animal was given 2 cc. of a culture of the globoid bodies from the spleen intraspinally and 4 cc. intraperitoneally. The only result of this injection was to bring about a temporary increase of the ataxia. Recovery finally became complete.

While a definite reaction was obtained in this instance, there is doubt whether the symptoms really indicated the production of experimental poliomyelitis.

Experiment E.—Macacus rhesus. June 2. An intraspinal injection was made of 2 cc. of a mass M.A. culture in the fourth generation. June 7. The animal was somewhat ataxic and excitable, and the right arm was protected. June 18. The animal was distinctly weak and tended to fall to the left side. Etherized. The autopsy revealed a generalized tuberculosis of the viscera. A few tubercles were detected in the meninges.

The histological examination of the spinal cord, medulla, brain, and intervertebral ganglia revealed no poliomyelitic lesions. A miliary tubercle was present in the intima of a small vein in one of the ganglia.

In other words, aside from the symptoms suggestive of experimental poliomyelitis, no pathologic basis for the diagnosis could be obtained. The experiment should be regarded as negative.

If we review the results of the inoculation of monkeys with cultures of the globoid bodies, we must conclude that, while certain symptoms suggestive of poliomyelitis were sometimes produced, in no instance was the experimental disease, as determined by the presence of typical lesions in the nervous organs, actually set up. Our experiments confirm, therefore, the conclusion arrived at by Flexner and Noguchi, that it is the very exceptional cultures only which retain pathogenic power sufficient to cause infection in monkeys.

DISCUSSION.

The presentation of this work on the cultivation of the globoid bodies may be considered from several points of view. Perhaps the first point that should be discussed is that relating to the ultimate results achieved. The experiments show clearly, I think, that if the experimenter has suitable poliomyelitic tissues to work on and suitable samples of ascitic fluid, and if the inoculated tubes are kept under strict anaerobic conditions, and transferred at proper intervals, successful results, in some degree, will almost surely follow. In fact, the decision arrived at was to the effect that it may be possible to cultivate the globoid bodies from practically all cases from which suitable material is available. If, for example, the ascitic fluid available during the period of May 15 to June 1 had been suitable, the globoid bodies present in the fluid medium could probably have been grown in the semisolid medium. Because of this failure, I can report the cultures merely as suggestive instead of as positive. Similarly, in other experiments (Nos. 3 and 4, Table II), it is highly probable that growth in the semisolid medium would have taken place had the hydrogen-nitrogen jar been employed instead of the vacuum jar.

However, the introduction of the hydrogen-nitrogen jar has not removed the chief drawbacks of the method; namely, the personal factor of painstaking care and perseverance. At best the methods

are long, tedious, often discouraging, requiring now and again months of time, abundance of suitable material, and an exact technique in order to succeed even in one experiment. I do not consider that I have modified fundamentally the original Noguchi technique. I have devised an alternative method which seems to possess certain advantages. And yet I never succeeded in identifying the globoid bodies in the first generation, although the original investigators did so.

The chief difficulty encountered is the establishment of the strain. It would appear either that the more pathogenic of the organisms do not develop in the artificial cultures, or that when they develop they do so at the expense, as a rule, of their power to produce infection. In this respect they may be said to resemble *Treponema pallidum*, with which they share so many cultural requirements and immunological reactions, as has previously been pointed out by others (1, 11).

After having once become established and accustomed to the artificial media, the globoid bodies grow more readily, may be more easily transferred, and will survive at refrigerator temperature for months. There are certain limitations, however, beyond which they will not go. Body fluids, preferably ascitic fluid, are required for their development, and strict anaerobiosis is also essential. Furthermore, the reaction of the culture media must be at approximately blood neutrality, and indicators, such as litmus, neutral red, Andrade's, etc., inhibit their growth.

The results obtained from the cultures of organs other than nervous tissues were unsatisfactory. The great obstacles to success with them appear to be contaminating organisms, particularly the streptococcus. This common microorganism appears to be more frequently present in the general viscera than in the central nervous organs; and the cultures prepared from the kidney and liver are the ones most often developing streptococci. Apparently also the globoid bodies are present less constantly or in smaller numbers in the general viscera. Finally, despite the exsanguination of the animal, the visceral tissues always contain a certain amount of blood, which probably interferes with the development of the globoid bodies in the initial culture tubes.

Despite these adverse factors, the successful cultivation of the organ-

ism from the spleen shows that it is contained outside the nervous and in the lymphatic visceral organs. Moreover, in four other instances, the globoid bodies were cultivated from the visceral tissues in the fluid medium, although they could not be developed in the semisolid medium. These strains included one from the adrenal of the animal yielding the spleen culture, another spleen culture, one from the kidney, and one from the mesenteric lymph node of two other monkeys. The experiments are the first reported in which the globoid bodies have been cultivated from tissues other than those of the central nervous system.

The inoculation of monkeys with the cultures should be regarded as having failed to produce the experimental poliomyelitis. The circumstances surrounding the failures are in themselves instructive. Had the deductions been based merely on the clinical symptoms, they would have pointed to the induction of the infection. The ultimate criteria of experimental poliomyelitis are (1) the typical histological lesions and (2) recommunicability of the disease by inoculation of the nervous tissues of the suspected case. Since neither of these could be satisfied in the experiments, I regarded them as negative.

CONCLUSIONS.

The globoid bodies, identical in morphological and cultural characteristics with the organisms described by Flexner and Noguchi, have been obtained in twenty-two cultures from the tissues of seven monkeys suffering from experimental poliomyelitis.

Twenty of the strains were cultivated from the central nervous organs, all being obtained from the cerebrum except one, which was cultivated from the cervical portion of the spinal cord.

Two strains were cultivated from the spleen.

None of the cultivated strains inoculated produced typical poliomyelitis in monkeys.

The recovery of a strain of the globoid bodies from the inoculated monkey is as difficult as is the original cultivation of the organisms from animals inoculated with the ordinary virus of poliomyelitis.

Nothing in this study has served to implicate the streptococcus in the pathology of the poliomyelitic process; the streptococcus is, how-

ever, encountered as a common contaminant or secondary invader, especially in animals which have been etherized while moribund, or which had died some hours previous to the autopsy. When the infected and paralyzed animals are killed while still strong, secondary invading bacteria, including the streptococcus, tend to be absent from the tissues.

A modified, perhaps improved, but alternative method has been devised for the cultivation of the globoid bodies and other microorganisms demanding a high degree of anaerobiosis.

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THE RELATION OF CIRCULATING ANTIBODIES TO SERUM DISEASE.

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Though all phases of anaphylaxis have been studied in animals, comparatively few systematic investigations have been made upon the condition as it occurs in man. Moreover, different species vary in their susceptibility to artificial sensitization of foreign proteins as well as in their reactions to a second injection of protein, and hence it is highly essential when opportunities arise to study in detail the problems of anaphylaxis in man.

In this connection the essential difference between man and the experimental animals is that the primary sensitizing dose of serum in animals is not followed by any obvious symptoms, whereas in man it frequently results in serum disease. The object of this investigation was to determine what relation, if any, existed between the formation of antibodies to horse serum and the course of serum disease.

During the last few years we have had an opportunity to study this problem in twenty-five individuals who, for therapeutic purposes, have received various quantities of antitoxic or antibacterial horse serum. Of the twenty-five cases, twenty-one suffered from pneumonia and received repeatedly large doses of antipneumococcus horse serum intravenously.[1] The smallest amount administered to one patient was 5 cc., the largest 630 cc., and the average amounts varied from 180 to 360 cc. Of the remaining four cases, one of meningitis received 40 cc. of antimeningococcus serum intraspinally, one

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[1] This serum was kindly furnished by Dr. Rufus Cole of the Hospital of The Rockefeller Institute for Medical Research and was an antiserum for Pneumococcus Type I.

case of tetanus received 22.5 cc. of antitetanus serum intraspinally, and two cases of diphtheria each received 7.5 cc. of the antitoxin subcutaneously.

To demonstrate the antisubstances to foreign protein in animals and occasionally in man, three methods have heretofore been employed: first, the test for precipitins; second, the demonstration of anaphylactin by the passive transfer of anaphylaxis by the blood serum of the test animals to a normal guinea pig; and third, the presence of a specific skin reaction. Since the classic studies of von Pirquet and Schick upon serum disease (1), a number of investigations have been made upon the relation that one or another of these reactions bears to the development of serum disease, but without definite results as regards their significance.

Hamburger and Moro (2), who first studied the precipitin reaction in children following injections of diphtheria antitoxin, found that the reaction appeared during serum disease, and as the precipitin increased in concentration in the blood the precipitinogen or horse serum decreased. They concluded that the interaction of precipitin and horse serum in the circulation produced a poisonous substance that was responsible for serum disease. Von Pirquet and Schick could find no relation between the presence of precipitins in the circulation and serum disease. Lemaire (3) confirmed the results of Hamburger and Moro, and stated, further, that precipitins were not demonstrable unless serum disease followed the injections of antitoxin. Wells (4), in a study of the precipitin content of the blood of children to whom diphtheria antitoxin had been administered, found precipitin in the circulation occasionally before the onset of serum disease. During serum disease precipitin was present in low concentration but rose rapidly in concentration towards the end of the illness to fall gradually thereafter and eventually disappear. Weil (5) has made similar observations.

Regarding the development of anaphylactin during serum disease, little is known. The isolated observations of Rosenau and Anderson (6), Anderson and Frost (7), Achard and Flandin (8), and Weil (9) show that anaphylactin to horse serum may be transferred passively from man to guinea pig. Novotny and Schick (10), however, succeeded in demonstrating anaphylactin in the blood of only two of twelve children that had received injections of horse serum. Grysez and Bernard (11), on the other hand, were able to sensitize guinea pigs passively to horse serum with the blood drawn from eleven children 5 to 243 days after the injection of horse serum.

Doerr and Russ (12), Doerr and Moldovan (13), and recently Weil (14) have brought forth evidence to show that the precipitating substance and anaphylactin are one and the same, but since it has also been shown both by Doerr and Moldovan and by Weil that serum may transmit passive anaphylaxis and yet

show no precipitating properties, it is necessary in any investigation upon the development of antibodies in serum to take into account the presence of both these reactions. It is possible, moreover, that the anaphylactic antibody which these observers find in the precipitate formed when antigen and antiserum are brought together may not be identical with the precipitate but absorbed by it during the process of precipitation. That protective antibodies may be carried down from antiserum by precipitates is well known,[2] and it is possible that the same may be true of anaphylactic antibody.

The occurrence of the specific skin reaction during and after serum disease has been observed by Hamburger and Pollak (15), Michiels (16), and Cowie (17). Michiels found that the reaction could rarely be obtained during serum sickness though it appeared shortly after the cessation of the illness. Cowie, by making repeated intradermal inoculations of 0.5 cc. of horse serum in children who had received immunizing doses of diphtheria antitoxin, obtained a positive skin reaction in one case on the 4th day, in seven cases on the 5th day, and in nine cases between the 6th and 10th days after the injection of antitoxin.

In the present investigation the three methods of study already discussed have been employed, though in only four cases have they all been applied to the same individual. In order to determine the time of appearance and the relation of these reactions to serum disease, observations were made at short intervals before, during, and after serum disease in all cases. The curves of precipitin formation were studied in fourteen cases, the development of anaphylactin in fifteen, and the appearance of the skin reaction in fifteen cases.

The precipitin reaction was carried out as follows: To 0.5 cc. of horse serum undiluted and in dilutions up to 1: 100,000 in isotonic salt solution, the patient's serum was added in quantities of 0.5 cc. As controls horse serum was titrated against anti-horse rabbit serum and normal human serum in similar amounts, while sheep serum and beef serum were titrated against the patient's serum. The tubes were kept at 37°C. for 1 hour and then allowed to stand over night in the ice box, when the final reading was made.

In attempts to transmit anaphylactin from one species to another cognizance must be taken of the fact demonstrated by Weil (18) and by Lewis (19) that when two different antigens or foreign proteins are simultaneously injected into an animal the protein in excess

[2] For a discussion of this subject see Gay, F. P., and Chickering, H. T., *J. Exp. Med.*, 1915, xxi, 389.

may prevent either active or passive sensitization to the other protein. It has also been repeatedly observed in attempts to transmit anaphylaxis passively that small amounts of serum will give positive results when the experiment fails with larger quantities. These facts soon became obvious in the present investigation and, therefore, in each experiment a series of guinea pigs was injected with varying quantities of the patients' serum.

The blood drawn from the patient was centrifugalized immediately, the serum separated, heated at 56°C. for 30 minutes, and injected intraperitoneally into four to eight guinea pigs in doses varying from 0.1 to 3 cc. In the majority of instances four doses were employed: 0.1 cc., 0.5 cc., 1 cc., and 3 cc. After an interval of 18 to 30 hours an intravenous injection of 0.5 cc. of horse serum was given to each guinea pig. Definite symptoms of anaphylactic shock were required before the result was termed positive.

To test the skin reaction 0.02 to 0.05 cc. of 1:10 and 1:100 dilutions of horse serum was injected intradermally by means of a specially graduated glass syringe. In the normal individual these minute quantities of serum may occasionally give rise to an immediate reaction. This consists in the appearance within 30 seconds to 1 minute of a red areola 1 to 3 cm. in diameter which rapidly fades within the next 5 minutes and is succeeded by a white indurated wheal 5 to 7 mm. in diameter. This may persist for 15 to 20 minutes. Since the same reaction may be obtained with various fluids, such as isotonic salt solution and water, it was considered by us as a traumatic reaction. In the individual sensitized to horse serum the traumatic reaction may or may not occur. Under any circumstances the true reaction makes its first appearance in 10 to 15 minutes. At this time the original wheal rapidly grows, becomes firm, yellowish in color, often itches, and at the same time a red areola spreads from the margin of the wheal until after 20 to 30 minutes the wheal reaches 15 to 20 mm. in diameter and the areola 30 to 50 mm. in diameter. Within half an hour the reaction begins to fade and after 1 to $1\frac{1}{2}$ hours subsides.

To follow the development of this reaction intradermal injections of diluted horse serum were always made before the therapeutic injections of horse serum and at frequent intervals afterwards until

strongly positive reactions were obtained. As controls, sheep and beef serum in the same quantities and isotonic salt solution were employed.

Of the twenty-five cases which were studied twenty-one developed serum disease that was manifest by such characteristic symptoms as enlargment of lymph nodes, skin eruptions, edema, fever, arthralgia, and enlargement of the spleen. The disease varied from a mild form (Case 11) to a severe relapsing form extending over a period of 3 weeks (Cases 1 and 2). In three cases there were no evidences of serum disease (Cases 12, 14, and 23), and in one patient (Case 13) who received three intraspinal injections of tetanus antitoxin, there were no other signs than slight enlargement of the lymph nodes.

Precipitins.

Of the fourteen cases in which the serum was tested for precipitins, twelve developed serum disease. In all but one of these, precipitins could be demonstrated on one and usually several occasions. Once having made their appearance in the serum they persisted, as a rule, for days and often weeks. In one patient who received 520 cc. of horse serum intravenously in divided doses precipitins could be demonstrated on the 34th day after their first appearance in such concentration that 0.5 cc. of the patient's serum formed a precipitate with 0.5 cc. of a 1: 100,000 dilution of horse serum. The serum from another patient who received 630 cc. of horse serum in divided doses contained precipitins for 58 days. In most instances the patient was discharged from the hospital before the precipitin reaction had disappeared. In two cases precipitins were demonstrated on one occasion only.

In the two patients who escaped serum disease, precipitins could not be demonstrated in the blood at any time. One patient received 300 cc. of horse serum in divided doses, the other 240 cc.

Anaphylactin.

Of the fifteen patients whose blood was tested for anaphylactin, twelve developed serum disease. With the serum of all but one of the latter patients it was possible on one or more occasions to

transmit anaphylaxis passively to guinea pigs. As a rule, the presence of anaphylactin in the circulation was of short duration and positive reactions could be obtained only once or twice. A typical example is shown in the protocol in Table I.

In one instance, however, positive reactions were obtained on five

TABLE I.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. B.; lobar pneumonia. Feb. 6, 1916. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 7. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 17. Onset of serum disease continuing until Feb. 26.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.	
	gm.	*1916*			*cc.*	*cc.*			
1	357	Feb. 11	5		0.5	0.5	26 hrs.	+ Recovery.	+
2	348	" 11	5		0.5	0.5	26 "	No symptoms.	
3	300	" 11	5		3.0	0.5	26 "	+ Recovery.	
4	205	" 11	5		3.0	0.5	26 "	No symptoms.	
5	Positive control. Jan. 12. 0.1 cc. of horse serum intravenously.					0.5	31 days.	+++ Dead in 3 min.	
6	195	Feb. 17	11	½	0.1	0.5	27½ hrs.	No symptoms.	–
7	204	" 17	11	½	0.5	0.5	27½ "	" "	
8	200	" 17	11	½	1.0	0.5	27½ "	" "	
9	210	" 17	11	½	3.0	0.5	27½ "	" "	
10	420	Positive control. Nov. 18, 1915. 0.25 cc. of horse serum intravenously.				0.5	92 days.	+++ Dead in 3 min.	
11	235	Feb. 21	15	4	0.1	0.5	28 hrs.	No symptoms.	++
12	210	" 21	15	4	0.5	0.5	28 "	" "	
13	183	" 21	15	4	1.0	0.5	28 "	++ Very slow recovery.	
14	217	" 21	15	4	3.0	0.5	28 "	+++ Dead in 12 hrs.	
15	299	Feb. 27	21	10	0.1	0.5	25 hrs.	No symptoms.	–
16	357	" 27	21	10	0.5	0.5	25 "	" "	
17	348	" 27	21	10	1.0	0.5	25 "	" "	
18	310	" 27	21	10	3.0	0.5	25 "	" "	
19	Positive control.					0.5		++ Recovery.	

TABLE II.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. N.; age 12 years; meningitis. Dec. 11, 1915. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 12. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 14. Onset of serum disease continuing until Dec. 22.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	*1915*			*cc.*	*cc.*	*hrs.*	
20	218	Dec. 16	5	2	0.1	0.5	26	No symptoms.
21	234	" 16	5	2	0.1	0.5	26	" "
22	235	" 16	5	2	0.25	0.5	26	" "
23	219	" 16	5	2	0.25	0.5	26	" "
24	267	Dec. 18	7	4	0.15	0.5	39	No symptoms.
25	302	" 18	7	4	0.20	0.5	39	" "
26	242	Dec. 21	10	7	0.1	0.5	22	+++ Recovery.
27	197	" 21	10	7	0.1	0.5	22	+++ "
28	182	" 21	10	7	1.0	0.5	22	+++ "
29	216	" 21	10	7	1.0	0.5	22	+++ "
		1916						
30	205	Jan. 3	23	20	0.1	0.5	22	+(?) Recovery.
31	245	" 3	23	20	0.5	0.5	22	++++ Dead in 4 min.
32	170	" 3	23	20	1.0	0.5	22	++++ " " 2 "
33	250	" 3	23	20	3.0	0.5	22	No symptoms.
34	250	Positive control. Nov. 18, 1915. 0.3 cc. of horse serum intravenously.				0.5		++++ Dead in 2 min.
35	257	Jan. 18	38	35	0.1	0.5	20	No symptoms.
36	286	" 18	38	35	0.5	0.5	20	" "
37	227	" 18	38	35	1.0	0.5	20	+(?) Recovery.
38	297	" 18	38	35	1.0	0.5	20	+(?) "
39	305	" 18	38	35	2.5	0.5	20	+++ "
40	236	" 18	38	35	3.0	0.5	20	++++ Dead in 8 min.
41	335	Positive control. Dec. 7, 1915. 0.5 cc. of horse serum intravenously.				0.5		++++ " " 2 "
42	247	Feb. 2	53	50	0.1	0.5	20	No symptoms.
43	234	" 2	53	50	0.1	0.5	20	" "
44	265	" 2	53	50	1.0	0.5	20	++ Recovery.
45	218	" 2	53	50	3.0	0.5	20	+++ "

TABLE II—*Concluded.*

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	1916			*cc.*	*cc.*	*hrs.*	
46	199	Feb. 21	72	69	0.1	0.5	28	No symptoms.
47	252	" 21	72	69	0.5	0.5	28	+ Recovery.
48	202	" 21	72	69	1.0	0.5	28	++ "
49	188	" 21	72	69	3.0	0.5	28	+++ "
50	302	Mar. 9	89	86	0.5	0.5	22	No symptoms.
51	270	" 9	89	86	1.0	0.5	22	" "
52	315	" 9	89	86	3.0	0.5	22	" "
53	Positive control. Jan. 2. 0.5 cc. of horse serum intravenously.					0.5		++++ Dead in 4 min.

occasions from the 10th to the 72nd day after the administration of 40 cc. of horse serum intraspinally. Table II gives the protocols of these experiments.

In three cases the injections of serum were not followed by serum disease. Anaphylactin could never be demonstrated in the serum drawn repeatedly from these patients.

Skin Reactions.

In fifteen cases repeated skin reactions were made and in all of these a positive response was sooner or later observed. The skin reactions, unlike the temporary presence of precipitins and the fleeting reaction for anaphylactin, having once appeared, persisted and increased in intensity as long as the patients could be observed. The reaction was also observed in three patients who did not develop serum disease and whose blood serum showed no precipitin or anaphylactic antibody for horse serum. In these three cases the sensitiveness of the skin was much delayed in its onset. In the twelve cases that suffered from serum disease, the hypersensitiveness of the skin became evident on the 7th to the 15th day after the first injection of serum, whereas in these three patients who did not develop serum disease the first evidence of skin hypersensitiveness was observed in one on the 17th day, in one on the 33rd day, and in one on the 50th day, after the first injection of serum.

From these results it is evident that in the cases which we have studied precipitins and anaphylactic antibodies were much more likely to be found in the blood serum of patients who suffered from serum disease following the injections of horse serum than in patients who escaped serum disease. In the latter instances precipitins and anaphylactic antibodies were not observed. The skin reaction, however, made its appearance irrespective of the amount of serum administered or the method of administration and appeared whether or not serum disease developed.

The Relation of Antibody Formation to Serum Disease.

Further study was made in an attempt to determine whether there existed any direct relation between the formation of circulating antibodies and serum disease, and, if so, what this relation might be.

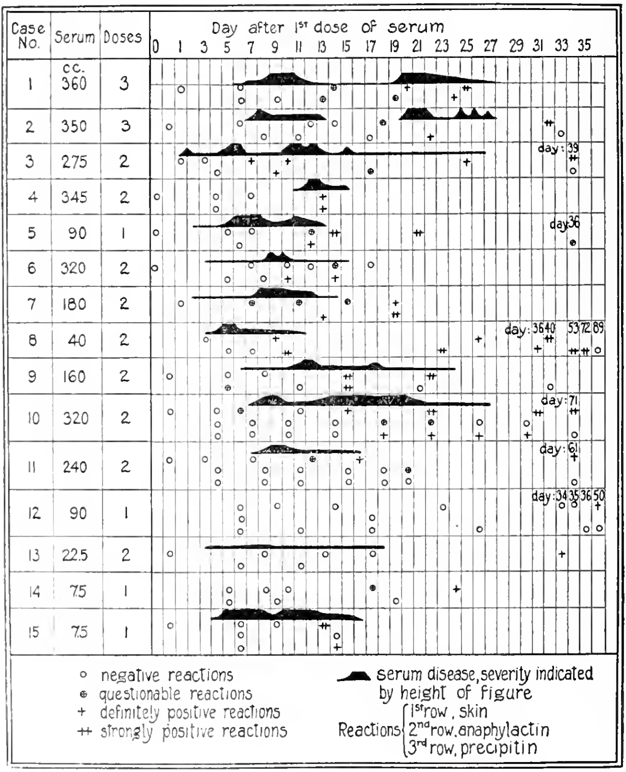
From the time that Hamburger and Moro demonstrated the presence of precipitin for horse serum in the blood of patients with serum disease, the question has constantly been raised as to whether these circulating antibodies play a part in the production of this illness. Von Pirquet and Schick were of the opinion that the illness depended upon the union of antibody and antigen either in the circulation or body cells and that the onset depended upon the proper balancing of these substances so that they might unite effectively to form a poison.

The problem may be viewed from three standpoints: (1) The formation of circulating antibodies may be totally independent of the serum disease. (2) The union of circulating antibodies and circulating antigen may produce a poison which gives rise to serum disease. (3) Antibodies may appear in the circulation as the result of a violent tissue reaction which manifests itself as serum disease, under which circumstances the antibodies might be protective rather than injurious.

The first proposition seems highly improbable, for otherwise one would expect antibodies to appear in the serum irrespective of serum disease, which was not true in this series of observations.

If the union of circulating antibody and circulating antigen were the sole cause of serum disease, one should be able to obtain evidence of the presence of these substances in the circulation immedi-

ately before or at the onset of serum disease. Such a demonstration has not been possible in these experiments. In only one instance (Table I) was a questionably positive result for anaphylactin obtained



TEXT-FIG. 1. The relation between the appearance of anaphylactin and precipitin in the circulation and of skin hypersensitiveness to serum disease in fifteen patients.

on the day previous to the onset of serum disease, and in no instance were precipitins present in the circulation before or at the time of onset of serum disease. Text-fig. 1 indicates the relation between the course of serum disease and the appearance of anaphylactin in the circulation.

It will be seen that blood serum drawn from the patients during the early stages of serum disease did not transmit anaphylaxis passively to guinea pigs. As a rule, anaphylactin was not demonstrable until the disease was well advanced or indeed about to terminate. This is particularly noticeable in Cases 1, 2, and 10, in whom relapses or recrudescences occurred. The blood from Case 1 did not give strongly positive results until the close of the relapse, which was 18 days after the onset of the disease. In Case 2 anaphylactin appeared 5 days before the close of the relapse and in Case 10 towards the subsidence of the recrudescence.

Table III is a composite table which shows even more strikingly

TABLE III.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Anaphylactin in the Blood and of Hypersensitiveness of the Skin to Horse Serum, in Twelve Cases.

Days after 1st injection of horse serum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	38	50
Onset of serum disease.......	1			2	1	1	4	1			2																		
Anaphylactin first demonstrated in blood serum..									1	2		1	3*		1			1		1†		1		1					
Recovery from serum disease.											2	1	1		2	2				1						1	2		
First positive skin reaction..							1		1			2	2	2	2			1	1				1‡					1‡	1‡

The numerals indicate the number of cases.

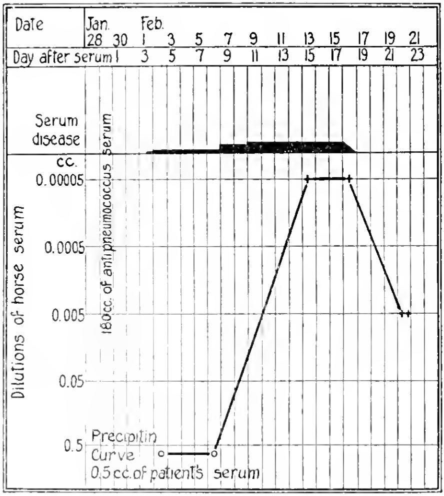
* One experiment doubtful but positive on 24th day.

† Doubtful reaction.

‡ No serum disease.

the average time relation between the appearance of anaphylactin and the course of serum disease. In Table III the average onset of serum disease came 4 to 8 days after the injection of serum, whereas anaphylactin makes its appearance 10 to 15 days after the injection of serum, and is more closely associated with the subsidence of serum sickness than with its onset.

Much the same relation was found to exist between the time of



TEXT-FIG. 2. The time relation of precipitin formation to serum disease. W. V. Type I pneumonia. 180 cc. of serum in two doses, January 29, 1917.

appearance of precipitin in the blood and the course of serum sickness in eleven cases that were studied. Precipitins were not demonstrable in the patient's blood serum until 3 or 4 days after the onset of serum sickness. After this time they rose rapidly in concentration to reach their maximum at the time of recovery. After recovery they diminished either rapidly or gradually in concentration. Text-fig. 2 is constructed from a typical protocol.

TABLE IV.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Precipitins in the Circulation in Eleven Cases.

Days after injection of horse serum.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	4	25	26	27	28	29	30	31	32	33
Onset of serum disease.....	1		1	2	2	1	1	1	1	1																							
Appearance of precipitins....									1	1	1	2	1	1	1	1		1	1														
Recovery from serum disease.		1										1	1		2		1		1	1	1					1							1

The numerals indicate the number of cases.

Precipitins were present in low concentration in the blood serum of three patients, in whom the serum sickness ran a protracted course, from the end of the 1st week to a period near the close of the disease when they rose rapidly in concentration. Wells and Weil have both made similar observations. On several occasions fever, which is often a late symptom of serum disease, was associated with the presence of precipitins in the circulation.

The composite Table IV, from the protocols of eleven cases, shows definitely the tendency for the appearance of precipitins in the circulation to precede immediately recovery from serum sickness.

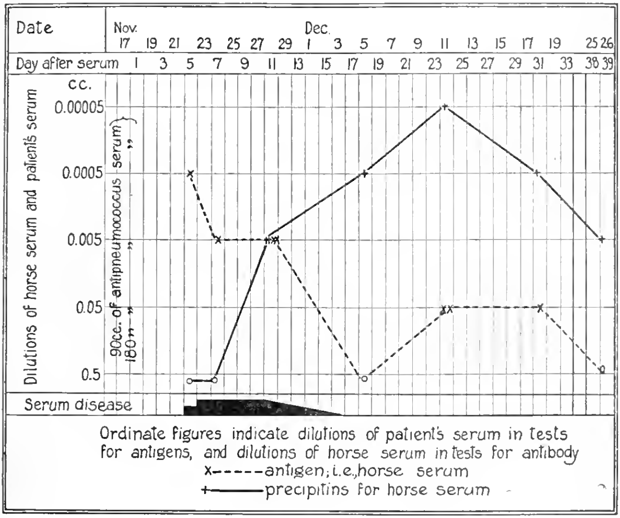
The relation of the appearance of the skin reaction to the course of serum disease in the cases that we have studied is made evident in Text-fig. 1. This reaction manifested itself only when serum disease was well advanced or had subsided, but since the skin became hypersensitive in all individuals who had received injections of horse serum and persisted over long periods of time, it seems probable that this reaction has a different significance from that of precipitin and anaphylactin formation, for the expulsion of these antibodies into the circulation is transient in character and definitely associated with recovery from serum disease, whereas the altered reaction of the skin is persistent and probably represents a changed characteristic of cells rather than a product of altered tissue reactions.

Our observations indicate a close relation between the appearance of antibodies for horse serum in the circulation and the termination of serum sickness. In no instance did antibodies appear either before or during the early stages of the disease, and in a few patients whose serum sickness was of unusually long duration there was a definite delay in the appearance of antibodies in the circulation. Further studies that have been made suggest that the appearance of antibodies in the circulation in great concentration is not only related to the termination of serum sickness but actually assists in bringing this about.

Early in the observations it became evident that circulating antibody to horse serum did not make its appearance unless it was preceded by a severe reaction in the tissue cells; namely, serum sickness. It seems highly improbable, therefore, that serum sickness depends upon a union of antigen and antibody in the circulation, but rather

that such a union, if it takes place, must do so, as Weil and others have frequently emphasized, within the cells of the body.

It is, however, obvious that antigen must be present either in the circulation or within the cells in order to bring about the reaction which we recognize as serum disease. Repeated observations have been made by us and by others to show that precipitinogen, or horse



TEXT-FIG. 3. Balance between antigen and precipitin in the circulation, and their relation to the course of serum disease. M. B., female; age 32 years. Type I pneumonia. Total 270 cc. of horse serum.

serum, remains in the circulation during at least a part of serum disease, so that both precipitin and precipitinogen are present in the circulation during serum sickness and for a few days thereafter. Most observers state that the antigen disappears more rapidly than precipitin, which remains in the circulation for days or weeks after recovery from serum disease. We have made an attempt to deter-

mine, by the methods of precipitation and the production of active anaphylaxis in guinea pigs, the exact time of disappearance of antigen from the circulation and the relation of its disappearance to the concentration of antibodies and to the duration of serum sickness. These observations, together with some others upon the absorption of antigen, will be reported later. Repeated observations have shown, as a rule, that there is a rapid disappearance of antigen upon the appearance of antibodies in the circulation in great concentration, and that the disappearance of antigen or the rapid diminution of antigen follows the rapid rise of precipitin and is coincident with recovery from serum disease. Text-fig. 3 is the reconstruction of a typical series of protocols showing the relation between precipitinogen, *i.e.* horse serum, and precipitin to the course of serum disease, and its termination.

DISCUSSION.

The experiments and observations recorded show that serum sickness is essentially dependent upon a reaction which takes place within the cells of the body and is probably dependent upon the formation of a toxic substance within these cells. Following this violent cellular reaction antibodies for horse serum are at first slowly and later rapidly extruded into the circulation in great concentration. As the antibodies rise in concentration the antigen rapidly disappears and coincident with this reversal in antibody-antigen content of the serum, serum sickness abates and the patient recovers. Should the antibody formation be slight in amount, the antigen may persist in large quantities in the circulation and the disease may be prolonged or may relapse.

We have shown that from the time of the primary injection of serum up to the onset of serum sickness, that is during the incubation period of this disease, the concentration of antigen in the circulation remains practically constant and antibodies to horse serum cannot be demonstrated. It seems highly probable, however, that during this period antisubstances are being formed within the cells and that at the time of onset of serum sickness, which is often explosive in character, there is a sudden union between the antibodies within the cells and circulating antigen which bathes the cells. The

course of serum disease depends at this time upon the production of antibody and the amount of available antigen. If antibody is formed in large amount and the quantity of antigen originally introduced is small, it is probable that the circulating antigen will rapidly be dispensed with and serum disease will be of short duration and mild in character. If, however, the amount of antigen originally injected is large and the formation of antibodies is slow and inefficient, it is highly probable that the available antigen will persist over long periods and that the serum disease will be severe in character and prolonged or relapsing in type. The production of circulating antibodies, therefore, seems a purely protective mechanism which is the method the body uses to destroy antigen, in this case horse serum. Since in serum disease the antigen is incapable of reproducing, the process can be accomplished, as a rule, rapidly and effectively, and recovery is prompt and complete.

Although we must always be cautious in drawing conclusions from analogies in biological experimentation, it is nevertheless admissible to suggest that these results may throw light on some of the methods by which the body rids itself of the injurious protein substances which represent antigen in an acute infectious disease.

CONCLUSIONS.

1. The injection of horse serum either in small or in large amounts in human beings is always followed sooner or later by the development of hypersensitiveness of the skin to subsequent injections of horse serum. For the development of this reaction serum disease is not essential.

2. The blood serum of most patients who suffer from an attack of serum disease following injections of horse serum shows anaphylactin and precipitin for horse serum.

3. Anaphylactin and precipitin cannot be demonstrated in the blood serum of patients treated with horse serum who do not later present symptoms of serum sickness.

4. The appearance of anaphylactin and precipitin precedes shortly recovery from the disease.

5. With the appearance in the serum of antibodies to horse serum in great concentration, the antigen rapidly diminishes or disappears.

6. It is probable that the extrusion of these antibodies into the circulation is the result and not the cause of serum sickness. Their presence serves to neutralize or destroy the antigen and thus determines the recovery from serum sickness.

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IMMUNITY IN INTESTINAL OBSTRUCTION.

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During the past few years a large amount of experimental work has been done in order to solve the problem of the cause of death in intestinal obstruction. This work has added to the knowledge of the physiology of the intestinal tract, but the cause of death from intestinal obstruction still remains unknown. It is generally conceded that a systemic bacterial invasion by the organisms of the obstructed intestine does not occur, and most of the clinical and experimental evidence so far obtained points to a quickly developing and rapidly fatal toxemia. The nature of the toxin is disputed, and investigations on this point thus far reported are not conclusive. The most extensive studies in this field have been made by Whipple, Stone, and Bernheim (1, 2, 3, 4), who ascribe the symptoms to a toxic primary proteose formed by the perverted activity of the intestinal mucosa. Leaving aside the question of how it is formed, we wished to determine, if possible, whether the toxic factor is really a primary proteose. A method of attack was suggested by the work done by Whipple, Stone, and Bernheim (3) in attempting to produce an immunity to the obstruction toxin. If an immunity to the toxin can be demonstrated it will show that the toxic factor in all probability belongs to that group of substances which have antigenic properties, namely the proteins, and their primary product of hydrolysis, the proteoses. The claim that a relation exists between antibody formation and non-proteins is apparently erroneous.

The conclusions of Whipple, Stone, and Bernheim,[1] as a result of their studies on immunity in intestinal obstruction, are not, in our opinion, warranted by the observations they have reported. Un-

[1] Whipple, Stone, and Bernheim (3), p. 164.

less extremely marked, immunity to a disease or toxin is always difficult to determine. In regard to the presence or absence of immunity in intestinal obstruction, we had noticed that dogs showed an extreme variability in their resistance to intestinal obstruction produced in various ways, and we believe that this normal variation recognized by Whipple and others accounts for most of their results.

The methods of Whipple, Stone, and Bernheim in localizing the immunity of a so called immunized dog should be noted. They obtained serum from a dog immunized by sublethal doses, added a lethal amount of duodenal fluid obtained from a closed duodenal loop, chloroform, and toluene, and incubated the mixture for 20 hours at 38°C. It was then tested, filtered, and injected in dogs. The injection caused death, and hence they concluded that there was no antiferment in the serum. Their further study was as follows:

> "Many of the immune organs were washed free from blood and ground to a paste which was combined with a lethal dose of the duodenal loop fluid, diluted with water to a thin paste, and allowed to autolyze at 38°C. for 2 to 5 days with chloroform and toluol. The filtrate was then tested on normal dogs by intravenous injection. The spleen and lung emulsion destroyed the poison. The liver juice (Buchner press), diluted and filtered, also destroyed it rapidly. The intestinal mucosa destroyed some of the poison but a part remained even after 5 days' digestion. A fresh mixture of liver juice and loop poison gave fatal intoxication, showing that the reaction is not prompt or does not take place in the blood stream. This result serves as a control. If the protective action resides in a single type of cell, it is the endothelial cell that may be concerned, but it is of course possible that various body cells may develop the reaction or produce the ferment."

Can we conclude that an immunity reaction is the cause of the loss of toxicity by a fluid of complicated composition allowed to autolyze 5 days under toluene and chloroform with organ extracts? It may be true that the liver and spleen would contain more antibody or a higher concentration, were there any formed, than the blood serum, but there would certainly be some liberated into the blood, and it is not probable that the intestinal mucosa and lung would contain large amounts of a neutralizing substance and the blood none. The fact that "fresh mixture of liver juice and loop poison gave fatal intoxication, showing that the reaction is not prompt or does not take place in the blood stream" would indicate that the reaction is not that between an antigen and an antibody. The statements that they discovered no neutralizing principle in the serum of immunized dogs and that they observed nothing of an ana-

phylactic reaction in guinea pigs detract from the evidence that the fluid has antigenic properties.

Davis and Morgan (5) were not able to confirm the results of Whipple and others, using autolysates of normal cat organs and cat serum with the fluid from dog closed loops, since they found that cats were apparently more highly resistant or immune to the intoxication of intestinal obstruction than dogs.

Nesbitt (6) endeavored to show that neurine may be split off from the choline in lecithin and is present in the intestinal canal during obstruction. He also noticed a ptomaine (unidentified) as a constituent of the material above an obstruction. Barger and Dale (7) demonstrated the presence of the depressor substance β-iminazolylethylamine in the intestinal mucosa. Mellanby and Twort (8) corroborated this and isolated a bacillus which they claimed could convert histidine into this substance. Murphy and Brooks (9) observed:

> "5. The toxicity of the loop content is not destroyed by heating to 60°C. until sterile, or even by boiling.
>
> 6. The toxicity of the fluid is very much decreased by filtration through a Berkefeld filter, so that to produce death a dose of filtrate corresponding to several times the lethal dose of unfiltered fluid is necessary.
>
> 7. The amount of filterable toxin is increased by prolonged autolysis."

Draper (10) was unable to find a proteose in the 1,000 cc. of loop fluid which he analyzed. These results all indicate that the toxic factor is not of a protein nature.

Methods.

As the symptoms caused by a closed intestinal loop, whether produced by ligature and a reconstruction of the gastrointestinal tract by gastroenterostomy, or by resection of the loop and an end to end anastomosis of the intestine, parallel closely the symptoms of acute intestinal obstruction, we have used the following control procedures: (1) the production of open intestinal loops, (2) the antemortem removal of closed intestinal loops, (3) the production of a blind duodenal stump, (4) ligature of the duodenum with no anastomosis, (5) injection of the material from closed intestinal loops.

Dogs were used in all the experiments. All operations were done under complete surgical anesthesia (morphine-ether) with the usual aseptic technique.

1. Production of Open Intestinal Loops.—As shown by Dragstedt, Moorhead, and Burcky (11), a certain proportion of dogs, in their work 50 per cent, can survive an open unwashed loop of the duo-

denum which is permitted to drain into the peritoneal cavity. This has been confirmed by the writers in a large number of dogs, and open loops have been made of the duodenum, jejunum, and ileum. While the number of dogs operated on in the lower part of the small intestine is not large, the work indicates that the lower the loop is, the smaller is the percentage of animals surviving an open unwashed intestinal loop—in approximate proportion to the increased number of bacteria found. The open loops upon later examination are in about half the instances found open and draining, while in the rest they are tightly closed by omental adhesions and are usually found fairly distended with a thick yellowish white material, which is often not sterile. Where the loops have been found open and draining a marked edematous and hemorrhagic appearance of the omentum and serous surfaces of the viscera is noticed, and three dogs died about 3 weeks after the operation from omental hemorrhage.

Whipple and his associates (4) produced an open loop of a different sort. The duodenum just below the pancreatic duct was cut across and ligated with inversion of ends and closure. A gastroenterostomy was done just below the duodenojejunal flexure so that a loop of the duodenum was produced which could drain into the jejunum. They state:

"The presence of such a partially isolated duodenal loop may be associated with intoxication, more or less severe, which will bring about an immunity reaction in the body cells. The intestinal mucosa from such a dog has the characteristic property of immune tissue; it can destroy with some rapidity the duodenal loop fluid *in vitro* and render the mixture harmless when given intravenously to a normal dog."

If there is a specific toxic secretion in a loop of such a nature that it can act as an antigen upon absorption, it is logical to conclude that the constant absorption of this substance would render dogs surviving open intestinal loops highly immune to the toxins of intestinal obstruction. All our methods of testing resistance to intestinal obstruction were tried out on these dogs, with the result given in Tables I, II, and X.

2. Antemortem Removal of Closed Intestinal Loops.—To answer the objection that an open intestinal loop is not an obstructed loop and hence the conditions necessary for the secretion of a hypothetical toxic proteose do not prevail, resected and closed unwashed loops of the beginning jejunum were produced in dogs and removed before the

loop had perforated, but as long after the production of the loop as the condition of the animal would warrant. The majority of the loops were markedly distended, already cyanotic in color, and contained approximately 80 to 110 cc. of bloody fluid. Although the period of immunization is necessarily short, it is to be expected that animals almost moribund from acute intestinal obstruction would, upon recovery, show a marked immunity if it can be produced. Here, too, there is tissue destruction, with resultant absorption of protein split products, and the chance of an increased absorption of the hypothetical toxic proteose. Hartwell, Hoguet, and Beekman (12) say that the toxemia is in proportion to the tissue necrosis, and without the latter there are no toxic symptoms. About half the dogs whose loops were removed before perforation died, indicating that there had been a marked absorption of toxic material by the time of the operation. Removal of the loop is a short and simple procedure. It was tried as an immunizing method in three series of dogs (Tables VI, IX, and X).

3. Production of a Blind Duodenal Stump.—Early in the work the authors noted that dogs in which a drained loop of the duodenum was made and the reconstruction of the canal effected by gastroenterostomy were not in as good condition as dogs in which end to end anastomosis was made. This was noted by Sweet, Peet, and Hendrix (13), and Whipple, Cooke, and Stearns (14) later operated to produce a blind duodenal stump as a method of causing a chronic type of obstruction in dogs. Most of the dogs in their series died in from 1 to 3 weeks with symptoms similar to those of obstruction. According to Whipple, these dogs have a definite tolerance to proteose injections. In conjunction with some other work the authors made a number of blind duodenal stumps, making the gastroenterostomy at the greater curvature and as near the pylorus as practicable, varying the length of the blind stump from about 8 to 50 cm.

If this type of operation results in a chronic obstruction and the toxin thereof is identical with the toxin of acute obstruction, this will be a better procedure to test out the immunity of a dog than the production of a closed loop as there is no perforation peritonitis to obscure results.

4. Ligature of the Duodenum with No Anastomosis.—In view of the

fact that the average length of life for closed loop dogs was higher in Whipple's series than in ours, a comparison of methods was necessary. The difference is easily explained. The closed loops as made by Whipple were produced by double ligature of the duodenum, just below the lower pancreatic duct and again at the duodeno-jejunal junction, reconstructing the tract by gastroenterostomy. This, of course, results in a longer loop than is possible by the method of resection and an intestinal anastomosis. The mechanical feature of this will, of course, explain the more rapid swelling of the smaller loop and an earlier death. Then there is the other factor, the cutting through of the ligature at either end of the loop by Whipple, permitting an escape of fluid from the loop, decreasing the tension within the loop, and thus preventing such a rapid swelling with consequent occlusion of the blood supply and necrosis of the intestinal wall as is found in the resected loop. This phase of the ligatured loop was studied extensively by the authors. The method consisted in ligating the duodenum with a single ligature, the size of which varied, and making no reconstruction of the canal, thus leaving the animal with an uncomplicated high obstruction. The ligature was buried with Lembert stitches. It was found that with a fairly heavy linen ligature the cutting through of the tissues and the restoration of the lumen begins in about 48 hours in the majority of instances, there being a lumen of about 1 to 2 mm. upon the 3rd day. Wide variations have been found. One dog showed a lumen of but 1 mm. after 11 days, while some showed two-thirds normal lumen after 48 hours. About 50 per cent of the dogs in which a ligature of the duodenum was done recovered completely. These dogs were tested for an immunity that they might have acquired as a result of the condition of acute obstruction which they had endured for about 48 to 72 hours.

5. *Injection of the Material from Closed Intestinal Loops.*—The last method of immunization was that used by Whipple; namely, the intravenous injection of the fluid from closed intestinal loops. The fluid was prepared according to the method of Whipple, with the exception that it was used within 2 or 3 days after preparation.

It is well known that many non-toxic substances, when kept in contact with such material as toluene and chloroform, may acquire a

certain degree of toxicity, and, aside from this, putrefaction is by no means prevented and any number of toxic substances may be formed which were not in the fluid at the time of collection. We were surprised to note the extreme variation in toxicity of different samples of fluid collected. 10 cc. in many instances have been fatal, while as much as 115 cc. of the undiluted fluid have caused no marked symptoms in other cases. Dogs that recovered from injections of this fluid were tested in the various ways outlined below for the existence of immunity or increased tolerance to intestinal obstruction.

Methods of Studying Immunity to or Tolerance to Intestinal Obstruction.

Production of Closed Intestinal Loops.—This is always a questionable procedure, owing to the fact that many dogs die from perforative peritonitis, to which, of course, immunity is impossible. The closed loops were made in the duodenum, as a fair proportion of the dogs die before the loop has perforated and in these dogs increased resistance can be readily observed. A dog in which the cause of death is perforative peritonitis can be easily observed after the production of the loop and the degree of resistance of the dog, in the earlier stages before the loop has perforated, noted.

Closed intestinal loops were made as a test procedure (*a*) in dogs which were strong and healthy after the open loop operation (Table I), (*b*) in dogs which had recovered from a ligatured obstruction (Table III), (*c*) in dogs from which closed loops had been removed (Table VI), and (*d*) in dogs which had previously been injected with closed loop fluid (Table VII).

Blind Duodenal Stump.—The question and method of production of a blind duodenal stump has been discussed above. Here there is no complication such as perforation and hence the results should be clear and indicative.

Blind duodenal stumps were produced in order to test the resistance (*a*) of dogs which had previously had open loops (Table II), (*b*) of dogs which had recovered from a ligatured obstruction (Table IV), (*c*) of dogs which had previously been injected with closed loop fluid (Table V), and (*d*) of a dog from which a closed loop had been removed (Table IX).

Injection of Closed Loop Fluid.—If the fluid contains the obstruction

toxin, then dogs recovered from obstruction should be more resistant to injections of it than normal dogs. On the other hand, if the fluid contains other toxic substances than that to which the dog may be expected to have increased resistance, there will be no immunity observed, following injection of the fluid, although the animal may in reality be immunized to the obstruction toxin. If, however, the other toxic substances present, as well as the obstruction toxin, are of such a nature that they can act as antigens, it is to be expected that injection of the fluid will markedly increase the resistance of the animal to the fluid. This should be true to an observable extent even if none of the toxic substances present besides the obstruction toxin have antigenic properties.

Is it not as logical to assume that if a number of toxic substances are found in the intestinal loop fluid the cause of death is due to all of them as it is to ascribe it to a particular perverted secretion, because the possible chemical nature of one toxin has been defined?

The resistance of dogs which had recovered from a ligatured obstruction, of dogs from which closed intestinal loops had been removed, of a dog which survived a closed washed intestinal loop (washing with sterile water and ether), of dogs with open intestinal loops, and of dogs which had received previous injections of loop fluid, to the injection of closed loop fluid, was compared with the resistance of normal dogs. The results are summarized in Table X. The results of injection of loop fluid into normal dogs is also included in this table to demonstrate the extreme variation in resistance that is met with normally.

Ligature of the Duodenum.—Although the percentage of recovery in normal dogs is comparatively high (about 50 per cent), it is to be expected that dogs immunized by any means to the toxin of obstruction would have such a resistance to the toxin that they would survive until the obstruction was relieved, by the cutting through of the ligature, to a much greater extent and in a greater number of cases than normal dogs. The percentage of recovery should be markedly increased. Our data on this point are not extensive for percentage results, but they are indicative.

Ligature of the duodenum was done as a test procedure only on a series of dogs which had received injections of closed loop fluid

(Table VIII). Four dogs of the seven survived over 5 weeks, which gives practically the same percentage of survival as in the control dogs given below.

Control Dogs.

Ligature of the Duodenum.—The duodenum just below the lower pancreatic duct was ligated in thirty-nine dogs with a linen ligature buried by Lembert stitches. In twenty-one dogs there was complete recovery. Seven of the remaining animals died in from 4 to 10 days from pneumonia contracted as a result of the toxemia and decreased resistance from the obstruction. The remaining eleven died in less than 96 hours with an uncomplicated autopsy picture.

Blind Duodenal Stump.—A blind duodenal stump was made in seventeen dogs by cutting the duodenum and in some instances the beginning of the jejunum, infolding the proximal end, and anastomosing the distal segment to the greater curvature of the stomach as close to the pylorus as practicable. The length of the blind stump varied from 10 to 65 cm. Two dogs are still living (3 months) and show no toxic symptoms. One died at the end of 2 months in extreme cachexia, the rest surviving the operation from 3 to 25 days. We found no direct correlation between the length of the blind stump and the degree of toxicity.

Closed Duodenal Loops.—The dogs in this series (twenty-six dogs) all died in from 24 to 96 hours, the average length of life being 48 hours. Nineteen of the loops were found to be perforated at autopsy. This gives a percentage of 73 dying from perforative peritonitis. If there is an increased tolerance in immune dogs, this percentage should be markedly increased and nearly all dogs would die as a result of the perforative peritonitis before the uncomplicated obstruction toxemia should prove fatal. None of the control dogs survived this type of loop in our series, although Sweet, Peet, and Hendrix report several instances in which the animal has lived for weeks. In the work of Dragstedt, Moorhead, and Burcky only two loops in six were found to be perforated. This gives a percentage of 33, but percentages from such a small series are misleading. Out of seven closed duodenal loops washed with water and ether, they found four perforated at autopsy.

TABLE I.

Open Loops with Later Production of Closed Loops.

Dog No.	Location of open loop.	Condition of open loop at 2nd operation.	Interval.	Location of closed loop.	Degree of toxemia preceding death.	Length of life.	Perforation.
			days			*hrs.*	
1	Duodenum.	Open.	36	Jejunum.	Toxemia after 24 hrs.	48	–*
2	"	Closed.	100	"	" " 15 "	19	+
3	"	Open.	33	"	" from start.	36	–
4	"	Closed.	21	"	" " "	60	–
5	"	Open.	26	"	Active until last few hours.	90	+
6	"	"	26	"	" " "	46	+
7	Jejunum.	"	62	Duodenum.	" " "	42	+
8	"	"	17	"	Toxemia from start.	40	+
9	"	"	24	"	" " "	26	+

* – indicates no perforation; +, perforation.

TABLE II.

Open Loops with Later Production of a Blind Duodenal Stump.

Dog No.	Location of open loop.	Condition of open loop at 2nd operation.	Interval.	Distance of blind end from pylorus.	Symptoms and remarks.	Length of life.
			days	*cm.*		
10	Jejunum.		16	38	No signs of toxemia.	Living (3 mos.).
11	"	Open.	16	42	Good recovery, then gradual decline.	7 days.
12	"		16	36	Cause of death unknown.	24 hrs.
13	"	Open.	8	45	Gradual decline.	5 days.
14	"	"	21	42	" "	5 "

TABLE III.

Dogs Recovered from a Ligatured Obstruction with Later Production of a Closed Loop.

Dog No.	Location of ligature.	Interval.	Location of closed loop.	Symptoms and remarks.	Length of life.	Perforation.
		days				
15	Lower pancreatic duct.	19	Duodenum.	Toxemia and recovery.	Living (3 mos.).	
16	" "	9	"	No toxic symptoms.	" (3 ").	
17	" "	10	"	Toxemia from start.	39 hrs.	+
18	" "	10	"	" " "	29 "	+
19	" "	9	"	" " "	32 "	+
20	Lower end of duodenum.	13	"	" " "	34 "	+
21	" "	13	"	Active until 4 hrs. before death.	56 "	+
22	" "	14	"	" " "	34 "	+

TABLE IV.

Dogs Recovered from a Ligatured Obstruction with Later Production of a Blind Duodenal Stump.

Dog No.	Location of ligature.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life.
		days	cm.		
23	Duodenum.	12	28	Cachexia and malnutrition.	25 days.
24	"	12	34	Pneumonia.	36 hrs.
25	"	10	38	Cachexia and malnutrition.	10 days.
26	"	9	30	" " "	20 "

TABLE V.

Dogs Injected with Closed Loop Fluid with Later Production of a Blind Duodenal Stump.

Dog No.	Times injected.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life
		days	cm.		
27	1	18	12	Gradual cachexia.	8 days.
28	2	9	35	" "	4 "
29	1	17	25	Lively.	Living (3 mos.).
30	1	17	36	Gradual cachexia.	5 days.

TABLE VI.

Closed Loops Removed and Second Closed Loops Made.

Dog No.	Location of 1st loop.	Interval before removal.	Condition at removal.	Interval.	Location of 2nd loop.	Symptoms and remarks.	Length of life.	Perforation.
		hrs.		*days*			*hrs.*	
31	Jejunum.	30	$\frac{4}{4}$*	28	Duodenum.	Toxemia from start.	34	+
32	"	21	$\frac{2}{4}$	19	Jejunum.	Mild toxemia from start.	48	+
33	"	20	$\frac{4}{4}$	15	Duodenum.	Active till 2 hrs. before death.	36	+
34	"	34	$\frac{3}{4}$	13	"	Toxemia from start.	30	+
35	"	72	$\frac{3}{4}$	7	"	Mild toxemia throughout.	38	−
36	"	48	$\frac{3}{4}$	6	"	Sudden onset of toxic symptoms 2 hrs. prior to death.	26	+
37	"	$34\frac{1}{2}$	$\frac{2}{4}$	40	"	Toxemia continuous.	48	−

* $\frac{4}{4}$ indicates distention to point of perforation; $\frac{1}{4}$ indicates beginning distention, etc.

TABLE VII.

Dogs Injected with Closed Loop Fluid with Later Production of Closed Loops.

Dog No.	Times injected.	Interval.	Location of closed loop.	Symptoms and remarks.	Length of life.	Perforation.
		days			*hrs.*	
38	1	27	Duodenum.	Mild toxemia.	36	+
39	1	12	"	" "	50	+
40	2	12	"	Active until last few hours.	38	+
41	1	8	"	" "	42	+
42	1	8	"	Toxemia from start.	28	−
37	1	12	"	" continuous.	48	−
43	1	7	Jejunum.	" "	24	−

TABLE VIII.

Dogs Injected with Closed Loop Fluid with Later Ligature of the Duodenum.

Dog No.	Times injected.	Interval.	Location of ligature.	Symptoms and remarks.	Length of life.
		days			*days*
44	3	7	Lower pancreatic duct.	Gradual toxemia.	7
45	3	7	" " "	" "	9
46	2	9	" " "	" "	5
47	1	20	" " "	Beginning toxemia after 24 hrs.	36
48	2	3	" " "	Complete recovery.	
49	1	4	" " "	Toxemia after 24 hrs.	48
50	1	5	" " "	Complete recovery.	

TABLE IX.

Closed Loop Removed with Later Production of a Blind Duodenal Stump.

Dog No.	Location of loop.	Condition at removal.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life.
			days	*cm.*		*days*
30	Jejunum.	$\frac{3}{4}$ distended.	34	36	Toxemia after 2nd day.	5

TABLE X.

Comparative Resistance of Dogs to the Injection of Closed Loop Fluid.

Fluid.	Dog No.	Condition of animal.	Weight.	Amount injected.	Amount per kilo.	Result.
			kg.	*cc.*	*cc.*	
W. R.	43	Normal.	6	6	1	Toxemia and recovery.
“	51	“	11	24	2.2	“ “ “
“	52	“	8.3	24	2.9	Dead in 5 hrs.
“	53	“	5.5	24	4.3	“ “ 8 “
“	16	Ligature and closed loop.	8	16	2	“ “ 18 “
A_2	29	Normal.	8	40	5	Good recovery.
A_2	54	“	8	80	10	Toxemia and recovery.
A_2	55	“	8	105	13	Dead in 10 hrs.
A_2	56	Closed loop removed.	6	33	5.5	Good recovery.
A_2	30	“ “ “	10	120	12	“ “
A_2	57	“ “ “	10.5	105	10	Toxemia and recovery.
404	46	Normal.	9.4	28	3	Good recovery.
404	58	“	11	33	3	“ “
404	38	“	8.9	27	3	Marked toxemia and recovery.
404	27	“	9	54	6	Good recovery.
404	59	Open loop.	6.3	19	3	“ “
404	44	Two previous injections.	10.4	31	3	“ “
404	45	“ “ “	12.5	38	3	Toxemia and recovery.
404	60	Open loop.	10.4	31	3	Marked toxemia and recovery.
404	46	One previous injection.	9.4	50	5.3	“ “ “
404	61	“ “ “	11	60	5.5	Dead in 6 hrs.
439	49	Normal.	7.7	45	5.8	Good recovery.
439	50	“	13	100	7.7	Toxemia and recovery.
439	48	One previous injection.	7.3	49	6.7	“ “ “
439	62	“ “ “	6	60	10	Dead in 12 hrs.
XO2	47	Normal.	13.2	33	2.5	Good recovery.
XO2	63	“	6.4	32	5	“ “
XO2	64	“	7.3	50	6.7	Dead in 8 hrs.
XO2	65	“	5.7	17.1	3	“ “ 8 “
XO2	66	“	13	98	7.5	“ “ 6 “
XO2	67	Open loop and closed washed loop.	8.6	27	3.1	Toxemia and recovery.
XO2	68	Open loop.	14.2	100	7	Good recovery.
XO2	69	One previous injection.	7	50	7.1	Dead in 4 hrs.
XO2	37	Closed loop removed.	10.7	32	3	Toxemia and recovery.
658	39	Normal.	17	102	6	Good recovery.
658	40	“	12	72	6	Toxemia and recovery
658	28	“	5	45	9	Good recovery.
27	70	Normal.	8	6	0.8	Dead in 19 hrs.
27	44	“	10.4	15	1.4	Toxemia and recovery.
27	45	“	12.5	5	0.4	“ “ “

DISCUSSION.

With few exceptions immunized dogs showed no greater resistance to subsequent obstruction than normal dogs, and in many instances they showed less. Two dogs that recovered from a ligation of the duodenum survived a closed unwashed duodenal loop, and are still living after 3 months. Upon later examination these loops were found to be only moderately distended and of good color. So far we have had no normal dogs survive a closed duodenal loop to this extent, but we are inclined to believe that the previous obstruction has altered the secretion-absorption ratio so that upon production of a closed loop there was no distention with consequent tissue necrosis, inasmuch as Sweet, Peet, and Hendrix report several instances of a normal dog surviving closed loops, and Dragstedt, Moorhead, and Burcky have shown that dogs can survive closed loops washed with ether. We do not consider that any immunity is shown by these cases. One dog immunized by injection survived a blind duodenal stump indefinitely. Controls have done this, however, and this is, therefore, no indication of an increased resistance.

Of the twenty-nine closed loops produced in immune dogs, twenty-one were found to be perforated after death. This gives a percentage of 72 for dogs dying of perforative peritonitis, which is no higher than that in control dogs and indicates that the immune dogs have no greater resistance to the toxemia than control dogs.

There still remains the possibility of an increased tolerance to the poison of intestinal obstruction. It is well known that carrion-eating animals can ingest quantities of putrefying protein that would poison man. What is the nature of the resistance to the poisons, and is it possible that dogs recovered from intestinal obstruction might show a slight increased tolerance to a later similar condition?

Our experiments so far do not indicate an increased tolerance, but if there is a tolerance of slight grade, it would take a great many experiments to demonstrate it. If this should prove to be the case, we believe that our work warrants the statement that the increased tolerance is due to some variable factor, such as diminished absorption in that section of intestine which was affected by the obstruction, since an increased tolerance has not been noticeable in a great number of our experiments.

CONCLUSIONS.

1. There is no increased immunity or tolerance to intestinal obstruction after recovery from previous obstruction.

2. Dogs recovered from intestinal obstruction are not more resistant to injections of closed loop fluid than normal dogs.

3. Dogs injected with closed loop fluid are not more resistant to intestinal obstruction than normal dogs.

4. In dogs the normal variation in resistance both to intestinal obstruction and to the injection of closed loop fluid is large.

The authors wish to express their appreciation to Dr. A. J. Carlson for advice and criticism during the course of the experiments.

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THE ACTION OF ANTISEPTICS ON THE TOXIN OF BACILLUS WELCHII.

A Preliminary Note.

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The chemical sterilization of wounds has resulted in much work on the bactericidal action of the antiseptics in general use.[1,2] In a former paper we reported[3] on the solvent action of some of the chlorinated antiseptics on necrotic tissue, pus, blood clot, and plasma clot. The only evidence that these antiseptics may have a destructive action on bacterial toxins is, first, the clinical observations of Carrel and Dehelly,[4] who noted that in patients with infected wounds treated with hypochlorite there seemed to be an amelioration of the general symptoms which they thought might be due to a reduction in the amount of toxin absorbed, and, second, some experiments by Lumière,[5] who found that pus containing virulent organisms, *B. tetani*, *B. welchii*, streptococci, and staphylococci, and presumably also bacterial toxins, became innocuous after the addition of hypochlorite solution and did not cause symptoms or death in animals injected with the mixture. Control animals, injected with the untreated pus, showed typical lesions. A second series of animals was injected with candle-filtered specimens of pus before and after treatment with hypochlorite solution. The animals that received filtrate from untreated pus showed symptoms of toxemia, while those in which the filtrate from the pus-hypochlorite mixture was injected showed no toxic effect.

It seemed desirable to perform a series of experiments with a definite toxin which could be quantitatively measured and a suitable, susceptible animal as an indicator. Bull and Pritchett[6] have demonstrated

[1] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160. Dakin, H. D., and Dunham, E. K., *ibid.*, 1917, ii, 641.

[2] Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

[3] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

[4] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 31.

[5] Lumière, A., *Compt. rend. Acad.*, 1916, i, 365.

[6] Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

a toxin for *Bacillus welchii* which fulfills all the requirements of the so called group of soluble or exotoxins. They have standardized the virulence of this toxin, and confirmed the unpublished observation of Flexner[7] that the pigeon is highly susceptible to the toxin and that the lesions produced in this animal are similar to those observed in human cases of gas gangrene. As wounds infected with *Bacillus welchii* are frequently encountered in military surgery today, and as the antiseptics studied are used extensively on wounds of this character, it was decided to use the toxin of Bull and Pritchett and the pigeon as a very sensitive indicator of the relative toxicity of the various toxin-antiseptic mixtures in the series of experiments recorded here. Ten experiments were performed. Comparable results were obtained in all, and the three series recorded below are in every way typical.

Method.

Production of Toxin.—Virulent strains of *Bacillus welchii* were grown for 18 hours in the culture medium described by Bull and Pritchett,[6] which, briefly, is made as follows: To 10 cc. of 0.2 per cent glucose broth are added a few fragments of sterile rabbit muscle. Inoculations are made into this medium under a layer of sterile paraffin oil and the cultures incubated in a vacuum jar from which the air has been exhausted. After incubation, the fluid is centrifuged for 20 minutes at high speed and filtered through a Berkefeld N candle. The different lots of toxin produced in this way are found to differ considerably in potency. For example, 0.3 cc. of the filtrate used in Experiment 1 contained one fatal dose of toxin, while it required 1 cc. of the filtrate used in Experiments 2 and 3 to produce a similar effect. In all cases the toxic filtrate was titrated previously to its use to determine the smallest amount which would kill, in 12 hours or less, a pigeon weighing from 300 to 400 gm., and this amount was considered as one lethal dose.

Treatment of Toxin with Antiseptic.—Volumes of filtrate containing the required number of fatal doses were measured into Esmarch dishes. Horse serum, inactivated at 58°C. for 1 hour, was next added to the solutions in which it was used. Sodium chloride solution,

[7] Flexner, S., quoted by Bull and Pritchett.[6]

0.9 per cent, was added to the portions requiring additional volume, and the antiseptic to be tested was added last of all. The volume used for injection was kept constant in each experiment, with the exceptions noted below. The antiseptic was allowed to remain for 5 minutes in contact with the other substances to be injected, and then the entire volume was injected into the pectoral muscles of a pigeon. Before injection, the feathers were removed from the breast and the skin was washed with alcohol. The results of Experiment 1 are shown in Table I.

TABLE I.

Experiment 1.

Pigeon No	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result
	gm.			*cc.*	*cc.*	
1	410	1			3	Died in 12 hrs.
2	425	2	3 cc. of Dakin's solution. *			Lived.
3	425	4	3 " " " " *			"
4	425	4	3 " " " " *	1.5		"
5	410	2	3 " " phenol " †			Died in 10 hrs.
6	425	4	3 " " " " †			" " 5 "
7	240		3 " " Dakin's " *			Lived.
8	325		3 " " phenol " †			"

* Dakin's solution titrated 0.5 per cent sodium hypochlorite concentration (made from bleaching powder).

† Phenol solution, 0.25 per cent.

Experiment 1.—One fatal dose of toxin killed Pigeon 1 in 12 hours. 3 cc. of Dakin's hypochlorite solution, titrating 0.5 per cent sodium hypochlorite, protected Pigeon 2 against two fatal doses, and the same amount of the solution protected Pigeon 3 against four fatal doses of toxin. That blood serum will cause hypochlorite solution to decompose is well known, and that it will reduce the effectiveness of this solution, at least as a germicide, is shown by the experiments reported by Dakin and his coworkers.[1, 2] 3 cc. of Dakin's hypochlorite solution protected Pigeon 4 against four fatal doses of toxin, even in the presence of 1.5 cc. of horse serum. Phenol did not exhibit

any protective action. Pigeon 5, injected with a mixture of 3 cc. of 0.25 per cent phenol and two fatal doses of toxin, died in 10 hours. Pigeon 6, receiving the same amount of phenol solution but four fatal doses of toxin, died 5 hours after inoculation. Pigeons 7 and 8, injected with the antiseptics in the same amount and concentration as employed in the toxin-antiseptic mixtures injected into the other pigeons, survived, thus demonstrating that the antiseptics themselves were not toxic and could not have explained the death of Pigeon 5 in 10 hours and that of Pigeon 6 in 5 hours.

Experiment 2.—The results of this experiment, recorded in Table II, confirm those obtained in Experiment 1. Because the toxin available at this time was not so potent as that used in the first experiment, it was necessary to use greater quantities for injection. In order that the total volume of the solutions to be injected should not be increased above 12 cc. and that the relative concentration of the antiseptics should be of a degree comparable with those used in Experiment 1, it was necessary to concentrate them somewhat; therefore a triple strength Dakin's hypochlorite solution, titrating 1.5 per cent sodium hypochlorite concentration, and a 1 per cent phenol solution were used. The final strength of the sodium hypochlorite and of phenol in the injected mixtures was comparable with that of those of Experiment 1, inasmuch as the dilution was considerably greater. The results were the same as those recorded for Experiment 1 in Table I.

Experiment 3.—In this experiment the action of chloramine-T was contrasted with that of Dakin's hypochlorite solution and of phenol. The results shown in Table III were similar to those obtained when the hypochlorite solution was used and confirm those summarized in Tables I and II. They also show that chloramine-T is able to protect pigeons against at least three fatal doses of the toxin and that its action is still demonstrable when serum is previously mixed with the toxin and the antiseptic is required to act on it as well as on the toxin.

TABLE II.

Experiment 2.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
9	270	1			7	Died in 15 hrs.*
10	310	3	2 cc. of Dakin's solution. †		3	Lived.
11	310	6	2 " " " " †			"
12‡	310	6	3 " " " " †	3		"
13	275	3	2 " " phenol " §		3	Died in 15 hrs.*
14	200	6	2 " " " " §			" " 15 " *
15	240		2 " " Dakin's " †		6	Lived.
16	410		2 " " phenol " §		6	"

* Over night.

† Dakin's solution titrating 1.5 per cent sodium hypochlorite (triple strength).

‡ In this pigeon the volume injected was 12 cc., in the others 8 cc.

§ 1 per cent.

TABLE III.

Experiment 3.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
17	480	1			7	Died in 12 hrs.
18	470	3	5 cc. of Dakin's solution. *			Lived.
19	430	6	2 " " " " †			"
20‡	460	6	2 " " " " †	4		"
21	420	3	5 " " phenol " §			Died in 5 hrs.
22	500	3	5 " " chloramine-T " ‖			Lived.
23‡	500	3	5 " " " " ‖	4		"
24	320		5 " " Dakin's " †		3	"
25	450		5 " " phenol " §		3	"
26	310		5 " " chloramine-T " ‖			"

* 0.5 per cent sodium hypochlorite titration.

† 1.73 per cent sodium hypochlorite titration.

‡ In this pigeon 12 cc. were injected, in the others 8 cc.

§ 0.25 per cent.

‖ 2 per cent chloramine-T (equivalent to 0.5 per cent sodium hypochlorite).

DISCUSSION.

From the experiments outlined above it is apparent that Dakin's hypochlorite and chloramine-T solutions will destroy the toxin produced by *Bacillus welchii*. It has seemed more precise, for experimental purposes, to make the mixtures of toxin and antiseptic *in vitro*, but from the experiments of Lumière[5] and the clinical observations of Carrel and Dehelly[4] it seems possible that these solutions may exert a similar influence when used in the treatment of infected wounds. The fact that the detoxicating action was still demonstrable when the toxin was treated with serum before the addition of the antiseptic adds to the clinical significance of these observations, because the conditions then closely simulate those encountered when the antiseptic is applied to wounds.

Phenol solutions of a final concentration of 0.25 per cent exhibited no destructive action on the toxin, and all the animals injected with a toxin-phenol mixture succumbed in the 24 hour interval following inoculation.

The control pigeons (Nos. 7 and 8, Table I; Nos. 15 and 16, Table II; and Nos. 24, 25, and 26, Table III) always survived; the antiseptic substances in the quantities and concentrations used, therefore, were not of themselves lethal.

No attempt was made to determine the maximum number of fatal doses of toxin against which a given amount and concentration of antiseptic was able to protect, nor did we go into the question of the length of time that the antiseptic and toxin must be in contact before injection in order that detoxication may occur.

The pathology of the lesions in the pigeons that died in the above experiments was substantially the same as that described by Bull and Pritchett.[6] In those that did not die varying grades of local edema, congestion, swelling, and discoloration of the skin and subcutaneous tissue were observed. These lesions were never marked and in no instance did they approach those observed in the birds receiving injections which resulted fatally. Those receiving phenol alone showed slightly more marked lesions than those receiving the hypochlorite alone.

In no instance did the pigeons recorded "Lived" in the tables die in the interval of observation, which was at least 1 week and in most

instances 2 weeks or longer. Any possibility of retarded deleterious effects, therefore, from the toxin-antiseptic mixtures injected, is practically excluded.

Finally, it seems desirable to add that these observations are not recorded with the purpose of advocating the use of an antiseptic in the place of the specific antitoxin produced by Bull and Pritchett.[6] In human surgery the antiseptic treatment of infected wounds will doubtless be combined with specific serum therapy.[8]

CONCLUSIONS.

1. Dakin's hypochlorite and chloramine-T solutions will protect pigeons against multiple fatal doses of the toxin of *Bacillus welchii* when the antiseptic and the toxin are mixed *in vitro* and allowed to stand in contact for 5 minutes before injection.

2. The detoxicating action of the solutions is demonstrable also in the presence of serum.

3. Phenol solution, 0.25 per cent, has no such action.

We take this opportunity to thank Dr. Bull and Miss Pritchett for their help and advice in the production and use of the toxin.

[8] A comparison of the behavior of these antiseptics enables us to distinguish two groups. In one, the antiseptic while bactericidal possesses little or no destructive action upon the products of bacterial activity; of this group phenol is an example. In the other group, the antiseptic attacks chemically not only the bacteria but also their products and by an alteration or disintegration of the molecules of the latter alters their properties and renders them inert; of this group the chlorinated antiseptics are the most striking examples. This action of these chlorinated antiseptics is to be attributed chiefly, as pointed out by Dakin, to their affinity for the amino group of the protein molecule.

THE FACTORS CONCERNED IN THE APPEARANCE OF NUCLEATED RED BLOOD CORPUSCLES IN THE PERIPHERAL BLOOD.

II. Influence of Procedures Designed to Increase the Rate of Blood Flow through the Blood-Forming Organs—Hemorrhage and Infusion.*

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In a previous paper the authors (1) have shown that the appearance of nucleated red corpuscles in the peripheral circulation is independent of the increased blood flow produced by exercise or by vasomotor paralysis. A slight increase in the number of these cells may be obtained, but one which, when thoroughly analyzed, never gives the picture of a true normoblast crisis. In the same paper it was said that the experiments on increased blood flow were designed to control more radical perfusion experiments by means of which cellular extrusion from the marrow could be studied directly. The interesting feature of normoblast appearance is its relation to blood regeneration, its reliability as an index of new formation. Perfusion work upon the marrow cannot be carried through a long enough period to give direct evidence of blood regeneration through increased red cell counts. It is therefore necessary to make use of the appearance of nucleated red cells as indicating the operation of stimuli for regeneration, provided this appearance can be shown to be an acute and reliable reaction.

In addition to a further demonstration of the fact that increased blood flow through the bone marrow has in itself no influence upon

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normoblast appearance, the present paper is designed to give a thorough picture of the nucleated red cell crisis, its time of appearance, duration, relation to marrow hyperplasia, etc. It would seem that nothing could be added to the literature of secondary anemia brought about by large, repeated hemorrhages. We had hoped on undertaking work upon the problem of blast appearance to find data which would place the reaction with complete accuracy. But such data do not exist so far as we have been able to ascertain. Tables giving the rate of regeneration in relation to hemoglobin formation are numerous, but neither these nor other studies give adequate pictures of the relation of nucleated red cells to regeneration.

Rieder (2) in his study of leukocytosis gives one instance in which normoblasts were found in the blood of a dog 24 hours after hemorrhage. This statement is unaccompanied by control figures of blood composition before blood loss.

Koeppe (3) finds nucleated red cells 23½ and 48 hours after hemorrhage in two rabbits. Here again there is lack of thorough examination before operation.

Zenoni (4) attempts to ascertain whether the appearance of nucleated red cells in the circulation is a result of the decrease of the blood mass through hemorrhage, or whether it is a hematopoietic reaction. Four dogs, two rabbits, and five guinea pigs were used, and care was taken that no normoblasts were present in the circulation before hemorrhage. The guinea pigs and rabbits were bled once from the carotid artery in large amount and films were made from the subcutaneous blood immediately and several hours after the operation. In one guinea pig normoblasts were found after 20 hours, in the two rabbits after 48 hours, and in one dog, treated in the same way, after 18 hours. No saline infusion was given in these cases. The remaining three dogs were bled repeatedly at one operation, the blood being defibrinated and returned. One showed normoblasts in 1½ hours, another in 5½, and the last in 7½ hours. Throughout the entire work there are no tables indicating the number of specimens examined, nor is there any note upon the character of the anesthesia. The hemorrhage and return of defibrinated blood involved a protracted operation. We have found that ether anesthesia, unless exceedingly brief, will cause the appearance of normoblasts with the familiar leukocytosis which is associated with this anesthetic. We may therefore summarize Zenoni's work in the conclusions that simple hemorrhage in rabbits, guinea pigs, and one dog required a considerable interval in order to cause blast appearance, and that rapidly repeated hemorrhages with return of defibrinated blood in dogs caused these cells to appear quickly. We have never repeated these last observations of Zenoni's, since they seemed to us to involve many possibilities aside from those he conjectured. The procedure undoubtedly gives repeated closing down and opening up of the marrow vessels as the circulation is depleted and replenished. This, of course, means periods of decreased and possibly in-

creased blood flow, but unless done under sterile conditions, without defibrination and the consequent introduction of serum and hemoglobin with their possibilities of leukocytosis production and accompanying blast appearance, it seems to us to lack the controls necessary for conclusive deductions.

Lazarus (5) states that nucleated red cells do not appear in human blood until the 2nd or 3rd day after hemorrhage.

Von Willebrand (6) in a series of observations on posthemorrhagic anemia in dogs and rabbits makes the following comment:

> Nucleated red corpuscles were observed after venesection in all the specimens of blood examined, but it must be stated in confirmation of the work of Timofejewsky, and Schauman and Rosenquist, that similar cells were found in three out of nine normal animals examined—dogs and rabbits—prior to venesection, although in these cases the nucleated cells were not numerous, the maximum being 4 in 10 sq. mm.
>
> The cells were usually not present in large numbers after venesection. As a rule they were most numerous during the first few days following the operation, and then disappeared gradually from the blood. I have never been able to observe so numerous an invasion of these cells as von Noorden observed and described under the term "blood crisis."
>
> The exact time of appearance of the nucleated red cells after bleeding cannot be readily determined, inasmuch as these cells may be present normally in the blood. In Experiment 3 relatively numerous cells (21 in place of 2 before venesection) appeared as early as $8\frac{1}{2}$ hours after the operation. All the nucleated cells were of the normoblast type. In size they resembled normal, unnucleated blood corpuscles.

Bunting (7) has compared the blood picture in a rabbit rendered anemic through repeated hemorrhages with that of ricin and saponin poisoning. He found 15 normoblasts per c. mm. 24 hours after the first of two hemorrhages totalling 20 cc. After 26 hours, 10 cc. more blood having been taken on the 24th hour, the number had risen to 108 normoblasts per c. mm. In a second case 12 cells were found 23 hours after a hemorrhage of 45 cc. In neither of these instances were counts recorded at short intervals immediately following the blood loss.

EXPERIMENTAL.

Dogs only were used in the experiments and since, as will be seen, the observations were carried over a long period, particular care was taken to keep the animals in good physical condition. They were fed on a diet of meat, bread, and dog biscuit. Except for an attack of acute and fatal distemper in Dog 1 none of the animals showed illness or suffered loss of weight. In addition to the five dogs whose records form the basis of this paper, we have bled with immediate infusion and bled without infusion six other animals, following the egress of cells at $\frac{1}{2}$ hour intervals for from 8 to 10 hours after the

operation. As the results thus obtained coincide with those obtained with the five animals subjected to more or less continuous observation we do not include them specifically in any of the data presented.

Charts of three of the five dogs of this series are given in this paper (Text-figs. 1, 2, and 3). In the case of the first two, we did not at first realize the need for daily observations so that their records are incomplete, but as far as they go they amply confirm the published data.

It was our original intention to bleed and infuse, studying the blood composition before the operation and at repeated, short intervals after it Experiments with the isolated tibia of the dog showed that hemorrhage and immediate infusion, with consequent blood dilution, will increase the blood flow through the bone often fivefold. Could it be shown that the sudden increase dislocated marrow cells? We have already commented upon the fact (1) that if the marrow vessels of all mammals are as incomplete as those pictured for the rabbit, allowing the normal blood current to wander through a loose network of cells, there should be easy dislocation of such cells under the influence of a markedly increased blood flow. That the dislocation does not occur readily has been indicated by the literature cited, but not proved, because of lack of precise following of the blood changes. It is noticeable that in none of the experiments cited has there been hemorrhage with immediate saline infusion. It is a question how long blood volume restoration by intravenous salt solution lasts. But there is no doubt that for the 1st hour following hemorrhage and infusion the volume of blood passing through the bone marrow is greatly increased. Boycott and Douglas (8) have shown extremely rapid restoration of blood volume in rabbits subjected to large hemorrhages and not infused. Dogs apparently restore blood volume more slowly, though Hünerfauth (9) found no difference between dogs and rabbits. It is possible that this rapidity of restoration indicates the reason our experiments upon blast extrusion show no variation whether or not salt solution was given. There is a frequent small increase in nucleated red cells immediately after hemorrhage, determined apparently by the fact that all intravascular blood cells are in the active blood current at this time and the true blood composition can, therefore, be properly appreciated on examination of specimens from the capillaries.

Boycott and Douglas (8) emphasize the increase in speed of regeneration of hemoglobin which follows repeated hemorrhage and consequent extension of erythrocyte-forming tissue. The effect of hemorrhage in drawing immature red cells into the circulation has not been studied in animals which, because of marked hyperplasia, are known to have a large volume of cell-bearing tissue. The fact that normoblasts accompanying leukocytosis appear with peculiar readiness in children has been commented upon, but the literature is lacking in precise following of the reaction through a series of hemorrhages.

Technique and Immediate Effects of Hemorrhage and Infusion.

Unless otherwise designated blood specimens were taken by subcutaneous puncture after a brief period of exercise. In every case red cell counts, white cell counts, and two films were made, the latter being invariably stained with Wright's stain. Nucleated red corpuscles were enumerated per c.mm. on the basis of the number seen while counting 1,000 leukocytes in the films. All red and white cell counts were made by two of us.

It was found early in the work that the individual hemorrhages were better borne and could be made much more extensive if accompanied by sterile saline infusions. Consequently figures given for the amount of blood removed do not represent whole blood, since in the latter part of the hemorrhage much of the fluid taken out had just been introduced. Under ordinary circumstances after removal of from 200 to 400 cc. of blood an equal amount of salt solution was returned through the same cannula. Then followed another large blood removal and saline injection, and so on until in our judgment the danger limit had been reached. While this method proved most satisfactory for the purpose of protracted experiments when it was essential to run no risk of losing the animal, it obviously leaves the estimate of the size of hemorrhage to the blood counts. In nearly all cases morphine and cocaine anesthesia was used, ether being necessary but a few times.

All operations were done under sterile conditions. Two bleedings could usually be obtained from each external jugular vein, one from each of the carotid arteries, one from each brachial artery, and in

large dogs one from each saphenous artery. This progressive cutting down of the vascular bed never produced permanent ill effects and gave opportunity for ten hemorrhages, more than enough for our purpose. On several occasions we saw temporary circus movements and amblyopia after large hemorrhages, but prompt recovery followed.

Tables I and II illustrate the immediate effect of hemorrhage and infusion upon the nucleated red cell count in a normal dog (Table I), and in the same animal rendered hyperplastic by four hemorrhages occurring at intervals during a period of 2½ months (Table II). On

TABLE I.

Immediate Effect of Hemorrhage and Infusion upon the Nucleated Red Cell Count in a Normal Dog.

Dog 2; female; weight 8.9 kilos; October 21, 1916.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				
1	9.35	4,960,000	12,700	127	1st experimental day.
	9.48				Ether begun.
2	10.09	5,488,000	18,500	203	Specimen 2 taken just before bleeding and after 21 minutes of ether.
Average......		5,224,000		165	
	10.09–10.23				360 cc. of blood removed in one large hemorrhage; 660 cc. of salt solution returned.
3	10.23	2,776,000	10,300	144	Taken immediately after saline infusion.
4	10.45	3,200,000	8,000	288	Animal rapidly coming out of ether.
5	11.30	3,504,000	11,900	154	
6	12.15	3,816,000	18,200	273	
7	1.00	3,400,000	19,700	157	
8	1.45	3,392,000	20,700	227	
9	2.30	2,992,000	20,200	141	
10	3.15	3,192,000	20,800	166	
11	4.00	3,000,000	17,800	142	
Average......		3,252,000		188	

October 21, 1916, Dog 2 in two observations showed an average of 165 nucleated red cells per c.mm. After a hemorrhage and an infusion of salt solution which reduced the red cell count from 5,224,000 to 3,252,000, nine observations during a period of $5\frac{1}{2}$ hours immediately following the hemorrhage gave an average of 188 nucleated red cells per c.mm. of blood. This increase, which becomes relatively larger when considered from the point of view of proportion of nucleated red cells to total red cell count, is nevertheless too small, in our opinion, to be regarded as representing a real dislocation of cells from the marrow. We believe it to have been due to a profound stirring up of the circulation following the infusion, and to a consequent more even distribution of cells throughout the peripheral area—a phenomenon such as we have shown to occur with exercise (1). This belief is further strengthened by the figures given in Table II, taken from the same animal.

On January 15, 1917, Dog 2, rendered hyperplastic by four fairly severe hemorrhages, was again bled and infused. Two observations before hemorrhage showed no nucleated red cells. Twelve observations following the infusion and extending over a period of 6 hours gave an average of 5 nucleated red cells per c.mm. Ten of these twelve observations showed no blasts at all. This insignificant increase in an animal whose marrow was presumably richly provided with nucleated red cells, strengthened our belief that the increased rate of blood flow following hemorrhage and infusion is not a procedure which will dislocate cells from marrow.

Table III summarizes briefly the immediate results of numerous hemorrhages in Dogs 3, 4, and 1, giving the number of specimens taken before and after the hemorrhage on each experimental day, the number of hours after hemorrhage during which the observations were made, and the average red cell, white cell, and nucleated red cell count before and after each hemorrhage. These figures confirm those taken from Dog 2. In thirteen out of nineteen cases a slight increase occurred in the number of nucleated red cells following the hemorrhage and infusion; in five cases a slight decrease in the number of blasts followed the hemorrhage; in one instance there was no change.

TABLE II.

Nucleated Red Cell Count in a Dog Rendered Hyperplastic by Four Hemorrhages at Intervals during a Period of 2½ Months.

Dog 2 (continued); January 15, 1917.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				
					Animal bled and infused on Oct. 21, 1916, Nov. 10, Dec. 14, and Jan. 1, 1917.
	8.50				1.2 cc. of 3 per cent solution of morphine sulfate subcutaneously.
1	8.55	7,528,000	11,500	0	
2	10.20	7,096,000	15,800	0	
Average......		7,312,000		0	
	10.20–10.40				Bled 630 cc. from external jugular vein; 1,100 cc. of salt solution infused.
3	10.40	2,432,000	17,200	0	
4	11.00	3,120,000	13,000	0	
5	11.30	3,284,000	17,600	0	
6	12.00	2,912,000	19,100	19	
7	12.30	3,176,000	19,400	0	
8	1.00	3,216,000	26,800	0	
9	1.30	3,286,000	24,250	48	
10	2.30	3,028,000	27,600	0	
11	3.00	3,008,000	30,100	0	
12	3.45	3,056,000	25,800	0	
13	4.30	2,944,000	28,000	0	
14	4.45	3,072,000	30,800	0	
Average......		3,044,000		5	

The True and the Pseudocrisis and the Time of Their Occurrence.

We have just pictured the immediate result of hemorrhage and infusion. On inspection of Text-figs. 1, 2, and 3 it is evident that the slight increase in nucleated red cells occurring immediately after

TABLE III.

Immediate Effect of Numerous Hemorrhages upon the Red Cell, White Cell, and Nucleated Red Cell Count.

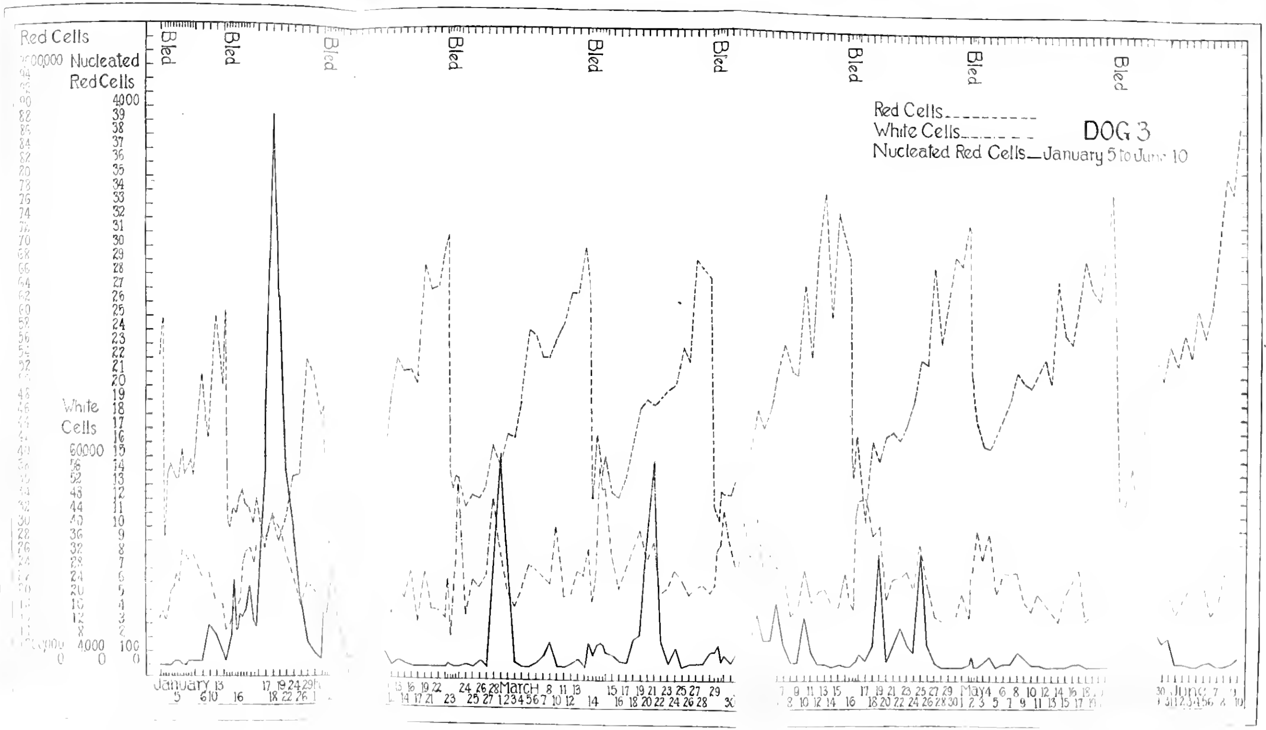
Date.	No. of specimens taken.		Duration of observation after hemorrhage.	Average No. of red cells per c.mm.		Average No. of white cells per c.mm.		Average No. of nucleated red cells per c.mm.	
	Before hemorrhage.	After hemorrhage.		Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.
				Dog 3.					
1917			*hrs.*						
Jan. 5	2	11	5¼	5,696,000	3,653,000	14,700	25,000	0	15
" 16	2	10	6	5,556,000	3,261,000	14,000	23,700	59	347
Feb. 2	2	6	5½	4,664,000	2,852,000	18,400	22,300	206	450
" 23	2	4	3½	7,122,000	3,720,000	19,800	29,600	12	2
Mar. 14	2	5	6¼	6,788,000	3,928,000	30,300	42,400	85	133
" 29	1	4	5¼	6,592,000	3,378,000	21,400	36,300	107	102
Apr. 16	2	3	5	7,040,000	4,058,000	23,200	36,300	0	57
May 2	1	3	4	7,416,000	4,834,000	14,400	32,700	14	26
				Dog 4.					
Feb. 9	2	6	5¼	8,812,000	6,384,000	17,000	21,200	0	0
" 20	2	6	5	6,113,000	5,214,000	20,700	38,200	0	266
Mar. 9	1	4	4¼	5,928,000	2,618,000	34,100	28,100	0	47
" 28	2	4	5	6,120,000	3,001,000	14,300	18,300	0	25
Apr. 17	2	3	5	6,772,000	3,320,000	15,100	30,500	14	55
May 4	1	2	2¼	7,040,000	5,463,000	17,800	28,300	0	52
				Dog 1.					
Mar. 7	1	6	5	6,072,000	4,042,000	28,300	18,000	28	2
" 16	1	5	5¼	5,018,000	2,724,000	18,000	19,400	54	65
Apr. 2	2	4	4¾	5,860,000	3,688,000	21,300	28,700	205	89
" 18	2	2	2½	6,158,000	4,080,000	13,600	16,700	423	245
May 7	1	1	½	6,124,000	3,360,000	16,600	8,900	33	320

hemorrhage never reaches a height comparable with that attained later in the curves, just before pronounced red cell increase. This first small addition of normoblasts is well seen in Text-fig. 1, Dog. 3, February 2, 1917. The result of hemorrhage and infusion on this day has been to bring into the active circulation every available cell. The situation is comparable with that seen on immediate exposure to low barometric pressure when polycythemia occurs at once and

expresses the full cell count of the animal at the moment. Later there is a formative polycythemia with the reestablishment of a reserve of red cells. In the same way, with the speeding up of the circulation which occurs immediately following hemorrhage and infusion, every available cell begins to circulate actively. The situation is exactly the one presented by exercise, and we designate the minor increases in nucleated red cells occurring after exercise and immediately after hemorrhage and infusion as pseudocrises. These pseudocrises do not foretell rapid regeneration and it is even doubtful in our minds whether the cells found in them really leave the marrow pulp. They are more probably in the circulation when the hemorrhage occurs.

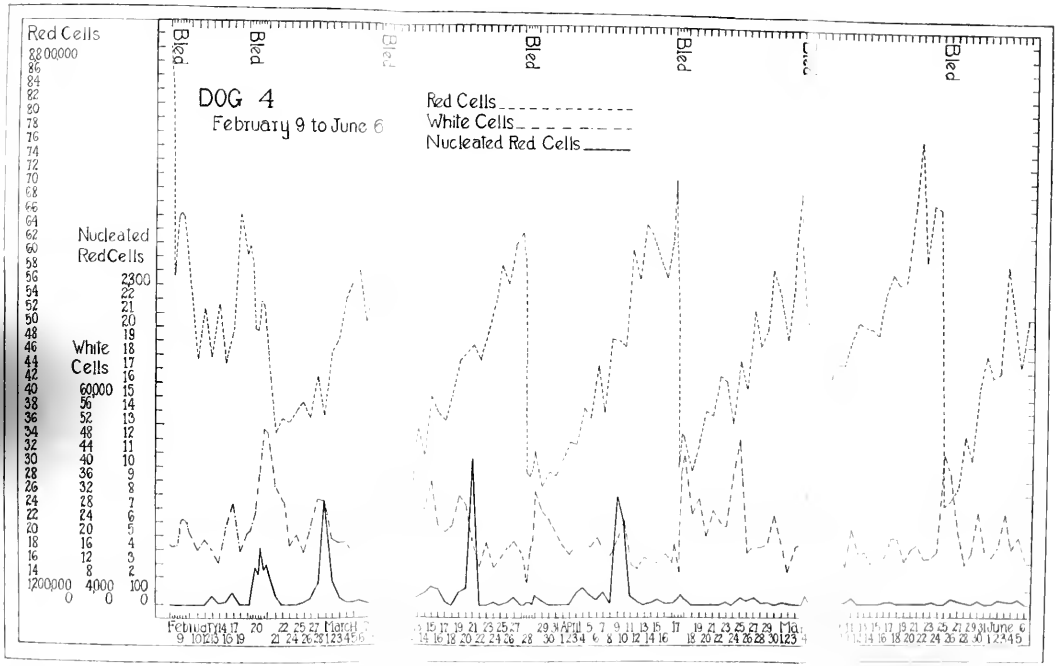
Other examples of the pseudocrisis are seen in Text-fig. 1, Dog 3, March 14 and 29. In these two instances the increases are less than on February 2 and they are preceded by periods of low normoblast content, while that of the earlier date is the result of hemorrhage just at the termination of an extremely large, true formative crisis. Even in the presence of the most advanced hyperplasia, evidenced by autopsy, the immediate effect of blood dilution and increased blood flow through the blood-forming organs is not to dislocate normoblasts but only to give a truer picture of the vascular content of these cells at the time of the operation.

True crises are easily recognized on the charts and their relation to red cell regeneration is obvious. They occur and disappear with great rapidity. This is particularly conspicuous in Text-fig. 3, Dog. 1. The first true crisis here begins on March 21 and ends on the 23rd; the second crisis, beginning April 13, lasts 3 days, in the first two of which the normoblasts increase from 434 to 8,210 per c.mm. It is plain that the crisis is always related to a favorable event, namely active red cell regeneration, but it is equally plain that the true formative crisis is not a necessary accompaniment of regeneration. Indeed, the transiency of the phenomenon, coupled with the fact that as animals are bled repeatedly they lose the tendency to show crises, emphasizes most strongly the fact that every effort is made to avoid this loss of nucleated red cells into the general circulation. A crisis usually occurs after the second hemorrhage. In the three charts presented this is marked. In two cases, Text-figs. 2 and 3, the crisis is largest after the third hemorrhage and then, with regeneration



TEXT-FIG. 1. Graphic picture of the daily red cell, wh ... and nucleated red cell counts of Dog 3 during a period of 5 months, showing the immediate and subsequent effects of nine ... ages. The ordinates represent cells per c.mm. of blood; the abscissæ re ... days of the month. When a number of observations were made on the same day immediately following a hemorrhage ... on, they are indicated by short vertical upstrokes on the line of the ... included between two long upstrokes, which indicate the ... and the last observation of the day. Single daily obser ... re indicated by single long upstrokes on the line of the abscissæ.

Contrast the pseudocrises of blasts immediately foll ... morrhage and infusion with the true blast crises occurrin during active red cell regeneration. Note the gradual devel ... of the ability to regenerate without crises and the steadily increasing ove ... tion of red cells as the hemorrhages continue.



TEXT-FIG. 2. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 4 during a period of 4 months, showing the immediate and subsequent effects of seven hemorrhages. The same features in the curve are to be noted as in Text-fig. 1. The initial high red cell count is probably due to fright polycythemia.

occurring rhythmically and rapidly after succeeding hemorrhages, there is a progressive decrease in the size of the crises. This gradual acquirement of a power to hold nucleated red cells in the face of rhythmic blood loss is an adaptation which might be expected. While dogs frequently show many nucleated red cells in the peripheral blood and in this case usually display the largest crisis after the first hemorrhage, they invariably begin to regenerate without crises as bleedings are continued. It is unfortunate that in our experiments the hemoglobin content was not followed in relation to this phenomenon, since the failure of hemoglobin regeneration at a rate comparable with cellular regeneration must produce a considerable color anemia in animals treated as these have been. The presence of a low hemoglobin has, however, had no effect on the rate of cell regeneration, and we may mention the fact that Neudörfer (10), one of the first to describe normoblast crises, observed them in cases of chlorosis.

A further question which naturally arises in relation to these sudden floods of nucleated red cells is: What is their fate? They disappear as quickly as they appear. As far as we know, their fate is entirely unexplained. Zuntz and his associates (11) believe that they are destroyed if present freely in the blood stream, but do not explain how. Our own observations, in which we have seen them resist the pounding of a mechanical glass perfusion pump through many hours without reduction in number, lead us to believe that they are as resistant to trauma as are non-nucleated cells.

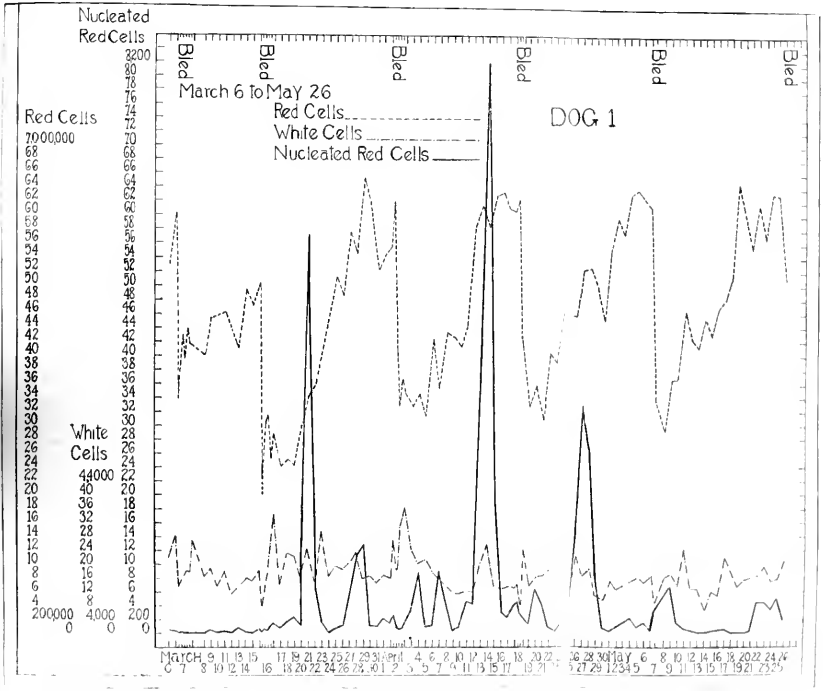
Concomitant Behavior of Red and of White Cells.

Those who have watched blood regeneration closely have been impressed with a tendency towards simultaneous movement of red and of white cells from the marrow. A high, sudden leukocytosis, polymorphonuclear in character, is frequently accompanied by a few nucleated red cells. Hough and Waddell (12) in studies of regeneration from single large hemorrhages in dogs state:

> "Each rise in the erythrocyte count is accompanied by a distinct rise in the leucocyte count either on the same or the preceding day. It is believed that, in general, when other causes of leucocytosis are controlled or absent, the leucocyte count may be taken as an indication of the degree of activity of the blood forming organs."

They do not report upon stained films, however, and have evidently observed increases in leukocyte count such as frequently accompany or just precede blast crises in our experiments. In the case of Dog 3, Text-fig. 1, the curves of nucleated red cells and leukocytes follow one another closely. Dog 4, Text-fig. 2, also shows increases in leukocyte count just before or with the blast crises of March 1 and 21 and April 9. However, during the period from April 12 to June 6, in which three large hemorrhages are given and recovered from, there are no normoblast crises, and with one exception no marked leukocytoses except those immediately after the hemorrhages. On April 24, 25, and 26 a definite leukocytosis occurs, caused by a localized abscess in the neck wound made in the bleeding on April 17. The white cells in this case have appeared in increased numbers and independently of normoblasts. Dog 1, Text-fig. 3, shows insignificant leukocytic increases with the blast crises, but nothing which alone would be significant as pointing to a regenerative tendency except possibly the leukocytosis beginning on April 13 and ending on the 15th. We may therefore agree with Hough and Waddell in feeling that a sudden and unaccountable leukocytosis may foretell rapid regeneration, but the reaction by no means always occurs before regeneration. Indeed, a tendency to avoid leukocytosis in the typical crisis position in the blood regeneration curve occurs as marrow hyperplasia advances. That the animals are well supplied with leukocytes is indicated by the vigorous posthemorrhagic leukocytoses which are seen to follow the hemorrhages throughout the entire course of the observations, and also from the readiness of development of the infectious leukocytosis in Dog 4.

Lastly, we should mention briefly posthemorrhagic leukocytosis. We have been impressed by the reliability and the extreme rapidity with which this reaction comes on. We have frequently seen the white count double in 2 hours. This is not a change in distribution of the white cells, since we have found the increase in specimens taken at the same time from heart, vein, and capillary blood. It is a real increase in the number of circulating white cells, and emphasizes the fact that the marrow is a reservoir of leukocytes in contrast to its relation to the red cells. Adult red cells are extruded minute by minute as fast as they are formed, and there is every tendency to



TEXT-FIG. 3. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 1 during a period of 3 months, showing the immediate and subsequent effects of six hemorrhages. Owing to the extremely large true blast crises which occurred in this animal the scale used in indicating the number of normoblasts is one-half the scale used for Dogs [illegible] and 4. The chief features in this curve are the same as those in Text-figs. 1 and 2.

resist undue haste in extrusion, as shown by the increasing power to hold nucleated cells which develops after a certain number of hemorrhages. But the marrow has an emergency function in relation to the leukocytes, a capacity to discharge the cells independently and rapidly, at times when many normoblasts are present, and under the influence of a procedure—hemorrhage—which brings about gradual increase in red cell counts.

Numerical Overregeneration of Red Cells and Influence of Repeated Hemorrhages on Rate of Regeneration.

Boycott and Douglas (8) have shown that after repeated bleedings in rabbits the rate of regeneration of hemoglobin becomes much greater than after a single hemorrhage, and that overregeneration occurs after single and multiple hemorrhages. The phenomenon of overregeneration of cells appears in our experiments. The high points in the curves become higher and higher as one passes to the right across the charts. This is particularly well seen in Dog 3, but in our opinion is equally true of Dog 4. The initial high red cell count in the latter animal was probably a fright polycythemia such as has been described by Lamson (13). The final regeneration after the bleeding was interrupted on June 6 by the utilization of this dog in a marrow perfusion experiment. Were it not for the fact that Boycott and Douglas have shown that there is a needless overregeneration of hemoglobin after hemorrhage, one might think that the polycythemic tendency which the repeatedly bled animals have shown was certainly due to the lag in hemoglobin formation, a multiplicity of cells carrying small amounts of hemoglobin taking the place of fewer cells better supplied. We followed this posthemorrhagic polycythemia for 2 months after the last bleeding in one animal, No. 2, upon whom, after six hemorrhages, all bleedings were discontinued. The red cell count at the end of this time was 9,336,000 cells per c.mm. This persistent polycythemia after repeated hemorrhages is comparable with the slow disappearance of the polycythemia of low barometric pressure. Apparently marrow hyperplasia once established resists disestablishment to a marked degree.

In considering the tendency to regenerate without crises, an adaptation of great interest, we have not met the criticism that such a process

may be due to marrow exhaustion. In all three animals whose records are given, autopsy with subsequent histological examination of the marrow disclosed marked hyperplasia, extension of erythrocyte-bearing marrow into the shafts of the long bones, and many islands of erythroblastic activity. Further, if one takes 6,000,000 cells per c.mm. as the normal red cell count of these animals, it is seen that regeneration to this level takes place in almost the same length of time throughout the entire series of bleedings. It is noteworthy that there is no significant increase in speed of regeneration as marrow hyperplasia advances, but that at the same time there is no lag in red cell formation. It has not been possible to confirm the work of Boycott and Douglas (8) on increased speed of hemoglobin regeneration by an analogous increase in speed of red cell regeneration. We have shown that after successive hemorrhages regeneration is not delayed, that the process apparently becomes more orderly as evidenced by the failure to find normoblasts in the peripheral blood, and ultimately reaches an abnormal height. Boycott (14) notes the fact that young rats and rabbits with hyperplastic marrow do not extrude nucleated red cells after hemorrhage as do adults. Our experiments with dogs do not entirely verify this since one of our animals, No. 1, a pup weighing 5.2 kilos when first obtained and 8.6 kilos at death $2\frac{1}{2}$ months later, showed large crises of normoblasts when first bled and passed into the state of regenerating without crises in much the same manner as did the adult dogs.

CONCLUSIONS.

1. Hemorrhage with immediate saline infusion causes the appearance in the peripheral blood of a slightly increased number of normoblasts provided normoblasts are already present in the blood stream. Marrow hyperplasia does not intensify this reaction and the cells found probably do not leave the marrow pulp but are in the blood stream at the time of the experiment.

2. The slight increase in cells occurring immediately after hemorrhage and infusion is designated a pseudocrisis. True crises are much more extensive; they tend to occur just before rapid increase in the erythrocyte count and usually towards the end of the 1st week following hemorrhage.

3. Red cells and white cells tend to move from the marrow together, but this association is not invariable.

4. After repeated hemorrhages regeneration occurs independently of the appearance of nucleated red cells in the peripheral blood.

5. Repeated hemorrhages associated with extension of erythrocyte-producing marrow lead to polycythemia but not to a conspicuous increase in speed of regeneration.

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THE MORPHOLOGICAL CHANGES IN THE TISSUES OF THE RABBIT AS A RESULT OF REDUCED OXIDATION.

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The experiments which are described in this paper were undertaken with the idea of determining the morphological changes which might be found in the tissues of animals subjected to the change of a single factor in their chemical environment. For this purpose two series of experiments have been carried out. In one, a decrease in oxygen of the respired air has been the sole factor of change. In the other, the change has been an increase in the carbon dioxide of the air with other factors kept as a constant.

In considering the results of the two series it would seem reasonable to assume that we are dealing with results, direct or indirect, in one case of a reduced oxygen supply to the cells of the body, and in the other of an increased carbon dioxide content of the fluids and tissues of the body. The assumption, however, that the morphological changes found could be produced in no other way is not made. Death of a cell may, for example, be produced by anemia, or by bacterial toxins. A finer analysis might, however, show that in both instances a reduction of vital oxidation was the cause of the lesion; on the one hand through oxygen starvation, on the other through failure to utilize oxygen by interference with the cellular oxidases by the toxin. It is our opinion, then, that oxygen deficiency should at least receive consideration as a cause, in interpreting lesions similar to those we shall describe in the animals subjected to low oxygen tension.

EXPERIMENTAL.

In carrying out the experiments we have used the respiration chamber described by Kolls and Loevenhart.[1]

[1] Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915–16, xxxix, 67.

In the first set of experiments ten rabbits varying from young to full grown were used (Table I). The duration of the experiments, in which the animals were exposed to low oxygen tensions, was 7, 10, and 4 days respectively. The animals were removed from the chamber not more than once during the progress of the experiment and then only during the time the chamber was being cleaned (about an hour). The number of hours during which the rabbits were subjected to different tensions of oxygen and carbon dioxide together with the

TABLE I.

Oxygen Rabbits.

Animal No.	Weight.			O_2		CO_2	
	Before experiment.	After experiment.	Loss.	Minimum.	Average.	Maximum.	Average.
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
1	2,100	1,820	280	4.78	7.74	0.80	0.25
4	950	820	130	4.78	7.74	0.80	0.25
9	1,900	1,380	520	3.58	7.21	0.30	0.23
10	940	720	220	3.58	7.21	0.30	0.23
11	1,320	1,000	320	3.58	7.21	0.30	0.23
12	2,080	1,700	380	3.58	7.21	0.30	0.23
13	1,300	987	313	5.47	7.33	0.20	0.20
14	1,660	1,419	241	5.47	7.33	0.20	0.20
15	760	Died.		5.47	7.98	0.20	0.20
16	1,680	"		5.47	7.98	0.20	0.20

Animal No.	Length of exposure to different O_2 tensions.*					Duration of experiment.
	12–10 % O_2.	10–8% O_2.	8–6% O_2.	Under 6% O_2.	Total.	
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*1915*
1	19.0	37.5	64.5	35.0	156.0	Oct. 18–25
4	19.0	37.5	64.5	35.0	156.0	" 18–25
9	11.50	86.25	73.0	60.50	231.25	Nov. 8–18
10	11.50	86.25	73.0	60.50	231.25	" 8–18
11	11.50	86.25	73.0	60.50	231.25	" 8–18
12	11.50	86.25	73.0	60.50	231.25	" 8–18
13		18.00	70.5	8.00	96.50	" 23–26
14		18.00	70.5	8.00	96.50	" 23–26
15		1.00	9.00	2.00	12.00	" 23
16		1.00	8.50	2.00	11.50	" 23

* Only O_2 tensions under 12 per cent are considered.

average tensions of the gases is recorded in Tables I and II. The carbon dioxide determinations were made with Haldane's portable apparatus for the analysis of mine gases. The oxygen determinations were made with a Hempel phosphorus pipette. At the termination of the experiments the animals were killed and immediately autopsied. The tissues were preserved in formalin, Zenker's solution, and 95 per cent alcohol. All the rabbits were weighed before they were put into the chamber and immediately before they were killed. A portion of the record of an experiment is given here.

Experiment 1. Reduced Oxygen.—Nov. 8, 1915.

Rabbit 9, white, male; weight 1,900 gm.

Rabbit 10, black, female; weight 940 gm.

Rabbit 11, speckled, male; weight 1,320 gm.

Rabbit 12, brown, male; weight 2,080 gm.

2.55 p.m. Experiment started. Burned hydrogen flame in the box to reduce oxygen rapidly, and started pump. All the animals were eating and seemed normal.

3.25 p.m. Rabbits becoming drowsy and slightly dyspneic.

3.30 p.m. O_2 = 9.61 per cent.

3.37 p.m. H turned off and O_2 turned on.

3.45 p.m. O_2 = 9.06 per cent. Animals becoming more dyspneic and drowsy. No. 10 seems to be most affected. Nos. 11 and 12 eating again.

4.15 p.m. O_2 = 9.11 per cent. Readjusted control for O_2 intake. Turned O_2 cup down slightly.

5.00 p.m. CO_2 = 0.25 per cent.

5.15 p.m. O_2 = 9.01 per cent.

7.15 p.m. O_2 = 8.47 per cent. Animals seem to be sleeping.

8.05 p.m. O_2 = 8.26 per cent. Readjusted O_2 intake.

10.40 p.m. O_2 = 8.65 per cent.

Nov. 9, 8.30 a.m. O_2 = 9.28 per cent. Readjusted O_2 intake. CO_2 = 0.30 per cent. Animals seem to be in good condition. Changed belt on motor.

8.50 a.m. Relative humidity 58 per cent.

9.00 a.m. O_2 off. The animals have been eating.

9.30 a.m. O_2 = 8.3 per cent.

11.50 a.m. O_2 = 6.16 per cent. Animals becoming very dyspneic.

1.05 p.m. O_2 on.

4.00 p.m. O_2 = 5.46 per cent. Readjusted O_2 intake.

4.05 p.m. CO_2 = 0.20 per cent.

4.15 p.m. Relative humidity 48 per cent. Drew off sulfuric acid.

7.00 p.m. O_2 = 7.12 per cent.

7.30 p.m. CO_2 = 0.25 per cent.

8.45 p.m. O_2 = 7.93 per cent.

The general data of the experiments are given in Tables I and II.

The symptoms manifested by the animals under different low oxygen tensions were constant in each series of experiments. When the oxygen was reduced to between 10 and 12 per cent, the rabbits became drowsy and slightly dyspneic. As the oxygen tension was reduced further, the dyspnea became more pronounced and was often accompanied by a swaying of the animal from side to side. When the tension was reduced below 7 per cent, the animals refused to eat or drink. Below 6 per cent oxygen, peculiar chewing movements were noticed. The movements of the rabbits became uncertain and it was difficult for them to retain the normal sitting posture. Inability to use the hind legs was noted in nearly all the rabbits. A characteristic symptom was the position of the head, which was thrown far back, possibly in an effort to make the act of breathing easier. One rabbit became paralyzed and appeared about to develop convulsions, when it was removed from the chamber. It recovered completely after a few minutes in normal atmosphere, but when put back into the chamber, the symptoms returned and the animal was discarded.

It is evident that three factors must be taken into account in interpreting the lesions: the low oxygen tension, the increased cardiorespiratory activity, and the lessened intake of food. The consistent loss of weight by all the animals indicates clearly that although the animals are not active in the cage, the intake of food is not sufficient for their vital demands. The general behavior of the animals indicates further that the compensatory effects, by the cardiorespiratory and hematopoietic systems, are not adequate to meet the severe demand made by the low percentage of oxygen which was maintained throughout the series of experiments.

The lesions of the animals at postmortem examination show the slight degree of change found in the various tissues. While the animals vary in the susceptibility of the different organs to oxygen deficiency there is a striking uniformity in the general picture. A single postmortem protocol may therefore suffice.

Rabbit 1.—Male; weight 1,820 gm. Killed Oct. 25, 1915.

Subcutaneous, mesenteric, and retroperitoneal tissues practically free from fat. Serous cavities free from excess of fluid.

Heart.—Large and pale. Left ventricle 5 mm. thick; right ventricle 1.5 mm. Muscle is uniformily pale on section.

Lungs.—Left: Pale and air-containing throughout and exudes a small amount of frothy serum from the lower lobe upon pressure. There are several punctate hemorrhages in the lower lobe. The border of the lung is slightly emphysematous. Right: Similar to left and also shows two small hemorrhages.

Spleen.—Measures 57 by 13 by 5 mm.; is of a deep purplish color and has a pitted and wrinkled surface. On section, the Malpighian corpuscles are of normal size. The pulp is increased in amount and is quite firm.

Liver.—Weight 59 gm. The capsular and cut surface are uniformly and finely mottled, due apparently to a dark appearance of the center and a light opaque appearance of the periphery of the lobule. The gall bladder is filled with dark green bile.

Kidneys.—Left: Measures 35 by 21 by 15 mm. Capsule is not adherent. The surface of the kidney has a uniform grayish pink color, which also characterizes the cortex on section. The medulla is paler. The cortex measures 4 mm. in thickness and has a regular striation. Right: Measures 34 by 22 by 12 mm. and resembles the left in every detail.

Adrenals.—Left: Measures 9 by 7 by 2 mm. On section, cortex measures 1 mm. in thickness. Medulla is linear and about 0.5 mm. in width. Right: Measures 10 by 7 by 2 mm. and is similar to left on section.

Pancreas.—Appears normal.

Aorta.—Intima is slightly opaque and appears thickened.

Thyroid.—Small and pale. External parathyroids small.

Bone Marrow.—From center of femur is of a deep opaque grayish red color, fairly firm, and gives no gross evidence of adipose tissue.

Brain and Cord.—No abnormality evident.

The tissues were fixed in Zenker's fluid, in 10 per cent formalin, and in alcohol.

Microscopic Examination.—

Heart.—Blood and lymph vessels throughout the section are distended and there is some edema of the tissues. The muscle fibers throughout are swollen, granular, and vacuolated, but the lesion becomes extreme only in the inner third of the heart wall. Here the fibers on cross-section appear to have but a single peripheral row of sarcostyles about a vacuole which contains a granular precipitate and in some cases is crossed by delicate fibrils. On longitudinal section the fibers are extremely thin but show the cross-striation. Many of the fibers show hyaline masses which extend usually across the width of the fiber but in some cases appear spherical and of a diameter less than that of the fiber itself. These hyaline masses occur both at the ends of the cells and at intervening points. The nuclei of the fibers stain sharply but appear somewhat shrunken. No fat is shown by osmic acid staining.

Lung.—The vessels are congested. The subpleural air spaces are excessively dilated and their walls appear thin. There are scattered minute alveolar hemorrhages, of antemortem occurrence as shown by the presence of occasional phagocytes containing red blood cells. There is slight edema. The lymphoid tissue is hyperplastic.

Spleen.—There is some hyperplasia of Malpighian corpuscles and of pulp cells as shown by mitotic figures. Both the pulp and its sinuses are distended with cells, chiefly red blood cells, although numerous polymorphonuclear leukocytes are found in the pulp. There are scattered nucleated red cells in the pulp, and in the sinuses are numerous groups of nucleated red cells of all sizes from megaloblast to normoblast, resembling the erythrogenic groups of the marrow.

Liver.—There is a general congestion of the vessels. The liver cells are most normal in the peripheral zone and next to the portal spaces. Here the cells are swollen and granular, with a tendency to a separation of the granules by ill defined clear spaces. Practically every cell has two nuclei. As one approaches the central vein, there is a tendency for the swollen cell to appear paler, to show a greater clear area crossed by an irregular protoplasmic reticulum, with scattered granules. In some parts of the liver clear-cut small fat vacuoles are found in the cells of the middle zone. In most of the sections the central zone shows necrosis of the liver cells and invasion by polymorphonuclear leukocytes. These areas of necrosis extend from central vein to central vein, about the periphery of the secretory lobule.

Kidney.—The vessels are congested. The cells of the cortical tubules are, in general, swollen until the tubular lumen is practically obliterated. The cells are granular but paler than normally. The granules appear to be separated from one another by serum. The lesion varies in the different cells from this condition to one in which the entire cell appears to consist of a nucleus and a vacuole surrounded by a cell membrane. In some cells the vacuole shows an irregular reticulum of protein material. The lesion appears most marked in the subcapsular region. Some of the tubules show a granular precipitate in the lumen and here and there a hyaline droplet is seen. There is no definite cast formation.

Bone Marrow.—The blood vessels are widely dilated and engorged with red cells. The marrow spaces are practically free from adipose tissue cells. The spaces are occupied by regular groups of nucleated red blood cells and by groups of myelocytes and leukocytes. The erythrocyte-producing centers predominate.

Aorta.—The muscle cells of the media are swollen and show a poorly defined vacuole about the nucleus.

Thyroid.—The alveoli of the thyroid are relatively small. The larger are filled with colloid, but there are many smaller alveoli devoid of colloid and practically without lumen.

Testicle.—There are few mature spermatozoa in the lumina. The germ cells appear to be vacuolated and in some places have pyknotic nuclei.

To summarize, the lesions found in the rabbit are: marked hyperplasia of the bone marrow; extramedullary (splenic) production of red cells; degenerative lesions of parenchymatous cells of the nature of a diminution of cell protoplasm with increase of serum (hydropic or vacuolar degeneration or serous imbibition); necrosis of parenchymat-

ous elements in some organs, especially the liver; hyaline degeneration and necrosis; congestion and edema of organs.

Rabbit 4, cage-mate of the animal described, showed practically the same lesions with two exceptions. The liver was less affected and showed no central necrosis, but instead well marked serous imbibition involving the whole lobule with the exception of an occasional cell close to the portal space. The kidney, on the other hand, gave evidence of a previous injury, in the presence of connective tissue scars, and appeared to be more injured by the oxygen deficiency. The serous imbibition was more marked, and some disintegration of cells and necrosis existed.

Rabbits 2 and 3 of this 7 day period were discarded as they were found to be pregnant when examined post mortem, although their lesions agreed with those described.

In studying the tissues of the rabbits of the 4 day and 10 day periods, similar lesions were found throughout, with but slight individual variations. In the hearts of animals of the 4 day period fatty infiltration was a prominent feature, and in fact was more marked than the vacuolar change. In the 10 day animals visible fat was lacking and the serous imbibition was the prominent lesion. In none of the four animals, however, was it as marked as in those of the 7 day period. This was interpreted as indicating a more complete though still insufficient compensation on the part of the blood and respiratory systems.

A tendency to emphysema of the borders of the lungs was noted in practically all the animals, though it was very slight in Rabbit 11. Minute hemorrhages and slight edema were constant. Hyperplasia of the lymphoid tissue in the lungs was also constant. In addition, in all the animals that were in the cages over 24 hours a mononuclear cell invasion of the lung framework of varying degree was present.

The livers uniformly showed hydropic degeneration of the cells of the mid-zone and central zone. In addition, Rabbits 12 and 14 showed a well marked central necrosis, while Rabbit 11 showed a fatty degeneration in the mid-zone and a serous imbibition in the central zone.

The kidneys of the series uniformly showed parenchymatous and hydropic degeneration. Hydropic degeneration was, however, very slight in Rabbit 14, which showed a well marked fatty degeneration.

The spleens showed, in general, hyperplasia of the Malpighian corpuscles and congestion of the pulp. Groups of erythroblasts were noted in the pulp sinuses in Rabbits 9 and 11. Rabbit 13 showed an excessive amount of hemosiderin in the pulp cells, a condition not found in the others of the series.

The aortæ of the animals generally showed a serous vacuolation of the smooth muscle fibers.

The bone marrow showed a marked hyperplasia in all the animals of the 7 and 10 day periods and less marked in those of the 4 day period. The erythroblastic centers were especially hyperplastic, although there was an increase also in the leukogenetic centers.

There was also a general hyperplastic condition of the thyroid gland in all the animals, with some variation in degree.

The lesions that we found then, in animals subjected to low oxygen atmospheres, may be summarized briefly as: (1) Hyperplastic changes in the bone marrow and in the thyroid. (2) Degenerative changes, with ultimate necrosis, in the elements of the heart and glandular organs. These changes are parenchymatous (albuminous), fatty, and serous (hydropic) degeneration, with some hyaline degeneration, and are most marked in the organs at points which are at the greatest distance from the blood supply; *i.e.*, the central zone of the liver, the inner part of the heart wall.

Before discussing the interpretation of the changes, it seems best to describe the experiments with animals kept at normal oxygen pressure and increased carbon dioxide. In this part of the work we have used only four rabbits, as the results were highly uniform. We find that there are certain similarities and differences as compared with the changes in animals subjected to diminished oxygen supply. Identical precautions were observed in this experiment with those in the series of reduced oxidation experiments in that normal atmospheric pressure, humidity, and temperature were maintained. The number of hours during which the animals were subjected to different carbon dioxide tensions is given in Table II.

The symptoms manifested by the animals in the atmosphere of high carbon dioxide were not so severe as those shown by the animals in the low oxygen atmospheres. The dyspnea was less marked, but the respirations had a peculiar short and jerky character. Through-

out the period, the animals ate and drank normally and showed no loss of muscular power, a condition contrasted to the first group described.

In examining these animals post mortem so little deviation from the normal was found, grossly at least, that it seems unnecessary to transcribe the protocols in detail. In fact, it would seem to suffice

TABLE II.

Carbon Dioxide Rabbits.

Animal No.	Weight.			O_2		CO_2	
	Before experiment.	After experiment.	Loss.	Minimum.	Average.	Maximum.	Average.
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
5	2,250	1,860	390	15.50	22.38	19.64	7.44
6	1,700	1,500	200	15.50	22.38	19.64	7.44
7	1,360	1,240	120	15.50	22.38	19.64	7.44
8	2,250	2,020	230	15.50	22.38	19.64	7.44

Animal No.	Length of exposure to different CO_2 tensions.										
	Under 1%	1-4%	4-6%	6-8%	8-10%	10-12%	12-14%	14-16%	16-18%	18-20%	Total.
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
5	4 0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
6	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
7	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
8	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5

The duration of experiment on each of the rabbits was from Oct. 28 to Nov. 4, 1915.

to point out how they differ from those of the reduced oxygen series.

Like the latter, the carbon dioxide animals lost weight and were found to have a minimum of body fat at the time of the postmortem. This occurred even though during the course of the experiment they appeared to eat normally.

The hearts showed no abnormality but the blood in the chambers was dark.

The lungs of the animals showed less emphysema of the free borders

than in the oxygen rabbits. Slight emphysema was noted in Nos. 7 and 8, none in Nos. 5 and 6.

The spleens of the animals showed a reduced amount of pulp which was also pale in contrast to the congested dark red pulp of the animals of the oxygen series.

The livers were of uniform color upon section and showed none of the fine mottling characteristic of the animals of the previous series, with the exception of Rabbit 7, which showed lobules with dark center and light periphery.

The kidneys were similar in appearance to those of the oxygen rabbits. Rabbit 5 showed many depressed cortical scars.

The most striking difference in postmortem findings was in the bone marrow taken from the center of the femur. The hyperplastic marrow of the reduced oxygen series was lacking. The marrow instead was soft, pale, and fatty. The absence of hyperplasia was fully confirmed by microscopic study. The bone marrows showed the presence of many adipose tissue cells and but a few peripheral groups of blood-producing elements.

The microscopic examination of the tissues of this series of animals showed a striking uniformity of lesions which were very slight in all cases and much less pronounced than those in the animals of the reduced oxygen experiments. The lesions were chiefly of a degenerative character and of the nature of the hydropic change noted in the other series.

The hearts uniformly showed, on section, muscle fibers which may be described as somewhat swollen, granular, and vacuolated (serous vacuolation). The vacuolation was never so extreme as in the earlier series. It showed, furthermore, a different distribution, being as well marked in the subpericardial layers of muscle fibers as in the deeper layers. Fatty infiltration was noted also in the hearts of Rabbits 5 and 8.

The lungs showed well marked congestion but no edema, very slight emphysema, and slight lymphoid hyperplasia.

The livers of the rabbits showed swollen, granular cells with a tendency to serous vacuolation. This was well marked in but one rabbit, No. 8, and in it was not of the degree seen in the oxygen deficiency rabbits. Moreover, in the four rabbits of the carbon dioxide series, the

change was more marked in the peripheral and mid-zone than in the central zone, the point of most marked change in the oxygen series.

The kidneys of the four rabbits showed a moderate swelling of the parenchymatous elements with increased granulation and some vacuolation. The last feature was well marked in Rabbits 6 and 8. The kidneys of Rabbits 5 and 8 showed old cortical scars and hyaline casts in the collecting tubules. Rabbit 7 also showed casts, and an albuminous precipitate in the glomerular capsular space.

The spleens showed well marked hyperplasia of the Malpighian corpuscles, and of pulp elements, with but moderate congestion. The spleen of Rabbit 5 showed an excess of hemosiderin in the pulp.

The thyroids showed a slight hyperplasia.

The aortæ showed slight fatty degeneration of the muscle fibers by a Sudan III stain, and serous imbibition was noted in the aorta of Rabbit 7.

The adrenals appeared normal.

The characteristic lesions in the rabbits exposed to a high carbon dioxide atmosphere may be summarized as very slight and of a degenerative character, affecting the parenchymatous cells of the heart and important glandular organs. This lesion which is of the nature of a serous imbibition, or vacuolar degeneration, is much less marked than in the animals exposed to an atmosphere deficient in oxygen and fails to show the same distribution, particularly in the heart and liver. Evidence of hyperplasia of the bone marrow is entirely lacking.

DISCUSSION.

In considering the results of these series of experiments, one finds in the oxygen deficiency series lesions of two types, those of a progressive or hyperplastic variety, and those of a retrogressive or degenerative character, while in the carbon dioxide series degenerative lesions alone are noted.

Another interesting contrast in the two sets of experiments is shown in the location of the degenerative lesions within certain of the affected organs. Thus in the oxygen deficiency experiments the lesion increases in intensity with increasing distance from the arterial blood supply. On the other hand, in the carbon dioxide experiments

the lesions are in general more marked the nearer the tissues are to the arterial supply. These facts are especially clearly brought out in the liver and in the heart muscle. The difference seems due to the fact that in one case we are dealing with a deficiency of a vital element in the arterial blood supply, whereas in the other we are dealing with a toxic agent reaching the tissues through the same medium.

The hyperplasia of the bone marrow, that is extension of the red marrow throughout the cavities of the long bones, found in the oxygen deficiency series is the most marked example of the progressive lesion. This finding is in accordance with the results obtained by Dallwig, Kolls, and Loevenhart[2] in animals subjected to the same experimental procedure as that employed here. This is a direct stimulation of the cells of the marrow to proliferation and differentiation by decrease in oxygen fixation of these cells and forms with the more prompt response of the respiratory center a good example of an adaptive mechanism of the body. The occurrence of degenerative processes in these animals would indicate, however, that the compensation was not adequate for the extremely low oxygen percentage maintained in the experiments.

Whether the hyperplasia of the thyroid may be looked on as the result of a deficiency in oxygen or as the result of some uncontrolled factor in the maintenance of the animals before or during the experiment must, perhaps, remain unanswered. Slight hyperplasia was noted in the thyroids of animals subjected to an increased carbon dioxide pressure, though not nearly so marked as in the oxygen deficiency series. If the theory that the thyroid is the gland controlling internal oxidation is maintained, such a hyperplasia would be expected in oxygen deficiency. Conversely, the finding of hyperplasia of the thyroid under these conditions may be urged as evidence in favor of the theory. It is to be noted further that this hyperplasia as a result of oxygen deficiency is in agreement with the marked hyperplastic state of the human thyroid which may be found in pernicious anemia where there is also a marked oxygen deficiency. Since it is well known that increased thyroid activity increases oxidation in the body it may be assumed, we believe, that in this

[2] Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915–16, xxxix, 77.

hyperplasia of the thyroid we have another adaptation to oxygen want.

The interpretation of the more general degenerative lesion in the rabbits of the oxygen series offers somewhat greater difficulty. The lesion is of the nature of an increase in the size of the cell with an increase in its fluid content and with apparently a decrease in the protoplasm, a lesion which has been termed hydropic degeneration, vacuolar degeneration, or serous imbibition. This lesion cannot be attributed to the inability of the experimenters to control the food intake in animals in the cages. The histological picture is entirely different from the simple atrophy found in young rabbits accidentally starved as a result of their having eaten sawdust or shavings, which accumulated in the stomach. Moreover, as pointed out, the lesion in the various organs is most marked at those points which are farthest from the blood supply, where in extreme cases anemic necrosis was found. The lesion appears then to be due definitely to a deficiency in oxygen.

Confirmation of this was offered by the tissues of a dog in the laboratory stock which had extreme stenosis of the trachea due to a marked goiter. A similar hydropic lesion was found throughout the parenchymatous organs.

It is of interest in connection with the theory of thyroid function, cited above, that the lesions found in these animals and attributed to oxygen deficiency are identical with the lesions described by Tatum[3] in animals which had been thyroidectomized early in life. Tatum notes that in this experimental cretinism "degenerative changes have been noted in practically every parenchymatous organ. Among these the most striking has been that of serous imbibition by the most active cells of these organs." Accepting the theory as to the function of the thyroid, Tatum's results confirm the interpretation we have given our results. Again, the converse is true. Our results, clearly due to changes in but a single factor, that of oxygen supply, explain Tatum's findings and support the contention that the thyroid is concerned in vital oxidation.

An attempt has been made by us to explain the morphological changes resulting from reduced oxidation upon the basis of physico-

[3] Tatum, A. L., *J. Exp. Med.*, 1913, xvii, 636.

chemical changes within cells. On this basis one might assume that as a result of reduced oxidation there occurs an increased osmotic pressure within the cell and a consequent increased flow of water into the cells. On the other hand, one might assume that as a result of suboxidation, an acid condition of the cell occurs which would in turn lead to acid imbibition by the colloids of the cells according to the theory of Fischer. It should be borne in mind that while decreased oxidation probably causes increased acidity, there is evidence that increased acidity leads to decreased oxidation. The experiments which we have performed do not enable us to determine whether the swelling of the cells was due to acid imbibition of the colloids or to the osmotic factor. The lesion itself as seen in the liver cell, for example, in the oxygen series is identical in appearance with that seen in the rabbit in moderate chloroform poisoning, in ether intoxication, and in extreme fatigue, as well as in the condition where the carbon dioxide content of the air is maintained at a high level. In the case of chloroform poisoning Graham[4] has drawn the conclusion that the pathological changes in the cell are due to the liberation of hydrochloric acid through the decomposition of chloroform, although he states in addition that chloroform is "a drug which is known to suppress oxidations." In carbon dioxide poisoning one must assume a cell retention of carbon dioxide with increased acidity, autolysis, and probably also suboxidation. In fatigue it is assumed that the edema of the cell is due to accumulation of acid waste products.

CONCLUSION.

Exposure of rabbits to an atmosphere of low oxygen content results in a stimulation of the cardiorespiratory systems, in an extension (hyperplasia) of red bone marrow and probably of a thyroid hyperplasia, with the further production of hydropic and hyaline degeneration in the cells of the parenchymatous organs. An atmosphere of high carbon dioxide and normal oxygen content produces, however, a stimulation of the cardiorespiratory systems, but no marrow extension and, in the concentrations used, but slight hydropic degeneration in the parenchyma of the glandular organs.

[4] Graham, E. A., *J. Exp. Med.*, 1912, xv, 307; 1915, xxi, 185, xxii, 48.

EXPERIMENTAL ACUTE NEPHRITIS.

A Study of the Acidosis, Nitrogen and Chloride Retention, and of the Protective Action of Sodium Bicarbonate.

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In an earlier study (1) it was shown that experimental uranium nephritis in the dog is characterized by an acidosis and an increase in the blood urea and plasma chlorides and that when sodium bicarbonate is administered daily the acidosis is of lesser degree, the increase of blood urea and plasma chlorides is not so great, and the lesions in the kidney are not so severe.

In the present investigation the scope of the work has been extended to include other forms of nephritis, those due to cantharidin, arsenic, diphtheria toxin, and potassium chromate (2). Inasmuch as the general literature on the subject was reviewed and the methods of study were described in detail in the first paper, these will not be again considered.

The observations were made on the dog and include the determination of (*a*) the carbon dioxide content of the plasma by the Van Slyke-Stillman-Cullen method (3), (*b*) the non-protein nitrogen of the blood by the Folin-Denis method (4), (*c*) the urea nitrogen of the blood by the Van Slyke-Cullen method (5, 6), (*d*) the chlorides of the plasma by the McLean-Van Slyke method (7), (*e*) the ketone of the blood by the Rothera method (8), (*f*) the hydrogen ion concentration of the urine by the Palmer-Henderson method (9).

All the animals were kept on a constant diet for several days and normal control determinations made before the production of the nephritis. All the control observations of blood and urine were made on at least 2 different days. In order to ensure definitely comparable results all bleedings for the purpose of obtaining blood for

chemical study were made at 9 a.m. and the animals were always fed at 3 p.m. All the nephritic poisons were injected subcutaneously at 9.30 a.m.

The observations fall naturally into two groups: (*a*) the purely chemical and pathological studies of nephritis and (*b*) the study of the influence of sodium bicarbonate. For the first group a severe nephritis was produced and for the latter a lesion of milder degree. The study of a severe nephritis due to each of the four renal poisons will be described before the studies illustrating the influence of sodium bicarbonate are presented. The milder degree of nephritis will be described later in connection with the study of the effects of sodium bicarbonate.

Cantharidin Nephritis.

The animals received cantharidin (1 per cent) (Merck) dissolved in acetic ether in the proportion of 0.002 gm. per kilo of body weight. Albumin and casts appeared promptly in the urine. The general results of the chemical study—an increase in non-protein and urea nitrogen and in chlorides and a diminution in the carbon dioxide con-

TABLE I.

Cantharidin Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.*		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 1	6.5	30.3	14.3	5.2	59	7.3	
" 3	6.9	32.1	15.6	5.7	60	7.6	
Mar. 4	Injection of 0.002 gm. of cantharidin solution in acetic ether per kilo of body weight.						
Mar. 5	6.3	55.8	28.6	6.2	43	6.9	Vomits; polyuria; refuses food.
Mar. 6	Comatose; died at 12 a.m.						

* The carbon dioxide content was determined by the original Van Slyke method (3).

tent of the plasma—are shown in Table I. These results are duplicated in the second dog receiving the same dose of cantharidin. In the second dog the changes were in the same direction but not of the same degree. Histologically the kidneys of these animals showed marked congestion of the blood vessels, extreme granular degeneration of the convoluted tubules, and plugs of granular material in the tubular lumina.

Arsenic Nephritis.

In this series, arsenic in the proportion of 0.002 gm. per kilo of body weight was given.[1] The dose caused the appearance of albumin and casts in the urine. The chemical changes were in the same direction (Table II) as in the other forms of nephritis. In a second dog

TABLE II.

Arsenic Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 20	6.3	40.8	15.6	5.5	53	6.3	
" 22	6.2	39.2	18.2	6.0	55	5.9	
Mar. 23	Injection of 0.002 gm. of arsenious acid per kilo of body weight.						
Mar. 24	6.0	55.9	23.4	6.6	48	5.6	Comatose.
	Died the night of Mar. 24–25.						

which was given arsenic the changes were of even less degree, and a return to normal was seen in the second 24 hours. Histologically the changes in the kidneys were limited to granular degeneration of the epithelium of the convoluted tubules with accumulation of granular material in the lumina.

[1] Arsenious acid (Merck) was dissolved in weak sodium carbonate solution, the solution neutralized to phenolphthalein, and distilled water added to make a 1 per cent solution.

Diphtheria Toxin Nephritis.

In this series the animals received diphtheria toxin of approximately $\frac{1}{150}$ toxicity, in the proportion of 0.02 cc. per kilo of body weight. Albumin and casts appeared in the urine and after 2 days the chemical studies indicated most marked changes. These are shown in Table III. Like results, except that the chlorides were slightly lower, were obtained in a second animal similarly treated.

TABLE III.

Diphtheria Toxin Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 8	6.8	36.2	11.7	5.6	54	6.8	
" 10	6.8	36.6	15.6	5.9	53	6.7	
						6.6	
Mar. 14	10 a.m. Injection of 0.02 cc. of diphtheria toxin ($\frac{1}{150}$) per kilo of body weight.						
Mar. 15	6.4	41.2	20.8	5.8	50	6.2	Refuses food.
" 17	6.2	156.2	86.1	6.4	31		Comatose. 10 a.m. Died.

Histologically the kidneys of these animals showed intense congestion and extensive granular degeneration of the cells of the convoluted tubules with occasionally hyaline droplets and here and there necrotic cells. Casts, both granular and hyaline, were found in the tubules. The glomeruli were shrunken and the capsular spaces occasionally contained a finely granular material.

Potassium Chromate Nephritis.

Potassium chromate (Merck) was dissolved in distilled water to make a 1 per cent solution. The chromate salt was given subcutaneously, first in the proportion of 0.006 gm. per kilo of body weight and after 4 days a second injection of 0.01 gm. per kilo. Albumin and casts appeared promptly in the urine and death occurred, preceded

usually by vomiting and a comatose condition (Table IV). These results are duplicated in the second dog. Histologically the kidneys of these animals showed the typical degeneration and necrotic lesions of chromate nephritis.

The results of the cantharidin, arsenic, diphtheria toxin, and chromate nephritis confirm our earlier work with uranium nitrate and show that in experimental nephritis there occurs, in addition to the retention of non-protein and urea nitrogen, a moderate acidosis. This is most marked in the diphtheria and chromate nephritis, but is evident also to lesser degree in cantharidin and arsenic nephritis.

TABLE IV.

Chromate Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Feb. 5	6.5	34.1	15.6	5.9	54	13.7	
" 6	6.4						
" 7	6.4	30.4	14.3	5.7	55	13.4	
Feb. 8	Injection of 0.006 gm. of potassium chromate (1 per cent solution) per kilo of body weight.						
Feb. 9	6.0	46.7	16.5	6.4	42	13.2	Refuses food.
" 11	6.3	88.6	28.2	6.1	46	13.1	" "
Feb. 13	Injection of 0.01 gm. of potassium chromate per kilo of body weight.						Refuses food.
Feb. 14	6.4	78.1	26.2	5.4	38	12.8	Refuses food.
" 15							Anuria.
" 16	6.5	118.5	41.6	5.7	36	11.7	Comatose. 10 a.m. Died.

Effects of Sodium Bicarbonate.

In the series in which the protecting effect of sodium bicarbonate was studied, a milder grade of nephritis was produced. Four dogs

were used for each type of nephritis and the experiments were conducted so that two animals in each experiment received the bicarbonate, while the other two, serving as the controls, received none. To all the same dose per kilo of the nephritic poison was given. The animals receiving sodium bicarbonate were given the bicarbonate for several days before the nephritic poison was administered and the bicarbonate administration was continued throughout the experiment. The sodium bicarbonate was given by stomach tube in the proportion of 2 gm. per kilo of body weight with 10 cc. of water for every gm. of the salt. This daily treatment followed the drawing of blood at 9 a.m., which was also the hour at which the nephritic poison was administered. All food was given at 3 p.m.

TABLE V.

Cantharidin Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 10	6.8	34.0	12.3	6.0	53	6.3	
" 11	6.8						
" 12	6.8	39.3	15.2	6.0	53	6.2	
May 13	Injection of 0.0015 gm. of cantharidin per kilo of body weight.						
May 14	6.8	37.0	18.2	6.3	40	5.8	Vomits.
" 15	6.5						"
" 16	6.5	61.4	31.2	5.6	38		Does not eat; comatose.
May 17	Died at 1 p.m.						

Cantharidin Nephritis.—A comparison of Tables V and VI shows the influence of the sodium bicarbonate in preventing the reduction of the carbon dioxide content of the plasma. The animals receiving bicarbonate, moreover, showed less tendency to vomit and did not refuse food. Histologically the kidneys showed an equal degree of nephritis whether bicarbonate had been given or not.

TABLE VI.

Cantharidin Nephritis. Sodium Bicarbonate.

From May 8, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 8		36.8	13.0	5.6	54	5.9	Soda commenced after bleeding.
" 10	8.4						
" 11	8.7						
" 12	8.6	32.7	12.8	5.4	59	5.9	
" 13	8.6						
" 14	8.7						
" 15	8.7	30.3	12.4	6.1	59	5.9	
May 16	Injection of 0.0015 gm. of cantharidin per kilo of body weight.						
May 17	8.6	45.9	16.9	5.9	57	5.3	Vomits.
" 18	8.7						
" 19	8.6	36.2	12.8	6.3	54	5.3	
" 20	8.6						
" 21	8.6	35 6	10.4	6.5	61	5.1	Chloroformed.

TABLE VII.

Arsenic Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 21	6.5	30 6	14 8	6.0	52	7.3	
Apr. 23	6.7	31.6	9.1	6.0	52	7.3	
	Injection of 0.0015 gm. of arsenious acid per kilo of body weight.						
Apr. 24	6.0	33.3	13.7	6.7	44	7.1	
" 25	6.1						
" 26	6.2	40.8	18.7	6.6	45	7.1	Chloroformed.

Arsenic Nephritis.—In this series the difference between the control and the bicarbonate dogs was slight, but was in accord with that occurring in the preceding series. The dog that received bicarbonate showed no diminution of plasma carbon dioxide. The nephritis in all was of a very mild grade. None of the animals had disturbances of digestion and no difference in the degree of histological changes in the kidneys could be recognized.

TABLE VIII.

Arsenic Nephritis. Sodium Bicarbonate.

From May 3, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 3	6.7	32.8	10.4	6.0	52	8.6	Soda commenced after bleeding.
" 4	8.3						
" 5	8.3	35.6	14.3	6.0	58	8.5	
" 6	8.5						
" 7	8.5						
May 8	8.5	Injection of 0.0015 gm. of arsenious acid per kilo of body weight.					
May 9	8.7	35.3	15.6	6.4	55	8.3	
" 10	8.5						
" 11	8.3	33.2	19.5	6.3	55	8.2	Chloroformed.

Diphtheria Toxin Nephritis.—Tables IX and X show the same difference in carbon dioxide content as in the experiments with arsenic and cantharidin, but digestive disturbances occurred in both bicarbonate and control dogs. Histologically changes in the kidneys of the animals receiving bicarbonate were of slightly lesser grade than in those of the controls.

TABLE IX.

Diphtheria Toxin Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood. Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.	Weight.	Remarks.
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 6	6.9	38.2	11.7	5.8	52	5.9	
" 9	6.9	40.2	13.0	5.5	52	5.8	
" 11	6.9	35.9	13.0	6 0	54	5.6	
Apr. 12	Injection of 0.015 cc. of diphtheria toxin per kilo of body weight.						
Apr. 13	6.7	41.6	13.6	6.6	45	5.2	Vomits; refuses food.
" 14	6.7	83.3	46.8	6.2	36	5.1	" 4 p.m. Died.

TABLE X.

Diphtheria Toxin Nephritis. Sodium Bicarbonate.

From April 6, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

Date.	Urine.	Blood. Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.	Weight.	Remarks.
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 6	6.8	36.4	10.4	5.6	51	5.8	Soda commenced after bleeding.
" 7	7.6						
" 8	7.8						
" 9	7.6	42.5	10.4	5.3	56	5.6	
" 11	7.8	39.3	9.1	5.8	60	5.5	
Apr. 12	Injection of 0.015 cc. of diphtheria toxin per kilo of body weight.						
Apr. 13	7.6	53.2	16.4	5.9	52	5.2	Vomits; does not eat.
" 14	7.6	62.2	46.8	6.2	53	5.0	"
	Died the night of Apr. 14–15.						

DISCUSSION.

The present work was undertaken to ascertain first what degree of acidosis occurs in experimental nephritis due to substances other than uranium, and second, how much protective action is obtained from sodium bicarbonate administered by stomach in these forms of nephritis It is clear that in the acute nephritis caused by cantharidin, arsenious acid, diphtheria toxin, and potassium chromate a moderate acidosis occurs, as shown by the diminution of the carbon dioxide content of the plasma and by the increased hydrogen ion concentration of the urine.

The non-protein and urea nitrogen and the chlorides in the blood, in the severe form, are always increased. In the milder grade of experimental nephritis, the action of the poisons was inconstant in the different dogs. While some dogs showed a moderate increase in the non-protein and urea nitrogen and in the chloride content of the blood; the others showed only very slight if any change. When sodium bicarbonate was given to these dogs the retention of nitrogen and of chlorides in the blood was in some instances less than in the control dogs but in other instances no difference could be noted.

In acute nephritis, a rise in the blood urea content may be due to renal insufficiency, and without doubt the impaired kidney function is to be considered the chief factor in the increase of the urea nitrogen in the blood, but such other factors as increased protein catabolism may be of some importance.

Gastrointestinal disturbances, indicated by the refusal of the animals to take food and by vomiting, frequently develop early in the course of the nephritis. Consequently the food intake of the nephritic dogs is not the same in different experiments and is not so constant as that during the preliminary control period. The possible influence of this change in food intake upon the catabolism must be considered, and it is not proper to attribute slight differences in the nitrogen content of the blood entirely to renal insufficiency.

Concerning the protective action of sodium bicarbonate against the acidosis it will be noted that the dogs receiving soda always showed a higher level of carbon dioxide content in the blood in the period before administration of the nephritic poison and, moreover,

that decrease in this figure during the nephritis was much less in the dogs receiving soda than in the controls without soda.

The hydrogen ion concentration was always diminished during the nephritic period in the control dogs, while in the dogs receiving soda it showed scarcely any change.

The most constant and striking difference between the control dogs and the dogs receiving soda in this mild grade of experimental nephritis was the less pronounced diminution of carbon dioxide content in the blood and the less pronounced decrease in the hydrogen ion concentration in urine in the dogs given soda, which indicates that the sodium bicarbonate has a protective action against the acidosis.

In some instances the animals when nephritic fell into a type of coma, being at first somnolent, then passing into a deep sleep, with deepened respiration. These symptoms are analogous to those seen in patients with uremic coma.

The association of severe acidosis and of coma is frequent. This suggested that there may be some relation between even the comparatively mild acidosis of these experiments and the greater tendency to coma that may be noted in the dogs receiving no soda.

Whether the acidosis of these types of experimental nephritis is due to a decrease of the excretory power of the kidney for acids, that is to retention of acids, or to the production of abnormal types of acids, or to increased production of acids is not known.

In all the animals acetone and acetone bodies were absent from the blood; hence, the acidosis in these types of experimental nephritis is not due to the acetone bodies.

CONCLUSIONS.

1. In experimental nephritis caused by cantharidin, arsenic, diphtheria toxin, and potassium chromate, in addition to the retention of the non-protein and urea nitrogen and of the chlorides in blood, an acidosis occurs.

2. Sodium bicarbonate given by the stomach has the power of diminishing the acidosis in these types of nephritis.

3. The degree of increase in the non-protein and urea nitrogen content in the blood in mild nephritis varies considerably in different dogs receiving the same dose per kilo of a given poison.

4. Histological examination shows little if any influence resulting from the administration of sodium bicarbonate upon the grade of the nephritis induced by the poisons.

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FURTHER STUDIES ON THE PROPERTIES OF PURE VACCINE VIRUS CULTIVATED IN VIVO.

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It has been shown that a sample of vaccinia virus, free from bacterial impurity and at the same time of sufficient strength for practical purposes, can be propagated in the testes of certain animals, particularly rabbits.[1] There were still several points, however, which seemed to require further investigation. It was not known whether or not, through continuous cultivation *in vivo* in the testicular tissues of rabbits, the biological properties of the virus would finally undergo modifications. Since the natural habitat of the virus is the skin, it is not improbable that the new medium in which it has been induced to grow might have a deleterious influence upon it. It was necessary to determine the viability of such a virus under varying conditions, that is at different temperatures, with the addition of antiseptics, etc. It was desirable also to compare the resistance and viability of the testicular strain with those of the virus propagated in the dermal tissue. These are questions not merely of purely academic interest, but of practical importance, inasmuch as the proper utilization of the new vaccine depends to a great extent upon the right understanding of its properties.

Scope and Mode of Experiments.

In the present series of experiments we studied the effect of disinfectants upon the testicular vaccine virus at different temperatures, the influence of diluents under various experimental conditions, the effects of desiccation upon the virus, and the viability of dried vaccine. The samples of testicular or of skin virus employed in each instance were highly active, being capable of producing a confluent eruption

[1] Noguchi, H., Pure cultivation *in vivo* of vaccine virus free from bacteria, *J. Exp. Med.*, 1915, xxi, 539.

on the shaved skin of the rabbit in 1: 1,000 dilution or higher. The vaccine preparations were placed in temperatures of 37°, 18°, 4°, and 0°C. Samples were taken from different test-tubes containing the virus at intervals in order to determine its strength under various conditions. During the 1st week the tests were made every 24 hours, since some of the vaccine samples which were kept at higher temperatures underwent a rapid attenuation of virulence, so that daily titration of their strength was imperative for following the course of deterioration.

The titration of the vaccine was made by applying a number of graduated concentrations of the specimen to a corresponding number of shaved areas, 10 by 20 cm., of the dorsal skin of rabbits. Special precautions were taken to have a control virus accompany the series of tests made on every animal in order to eliminate the irregular results due to individual variations in susceptibility to the vaccine virus, for without a proper control for each rabbit no accurate estimation of the vaccine effect may be made. In the present work duplicate tests were often resorted to. It was found both practical and reliable to make as many shaved areas as the dorsal and lateral surfaces of the rabbit's body permits, leaving narrow strips of hair between the shaved areas to serve as barriers, preventing the accidental overflow of one dilution to the next area. Readings of the results were made at intervals for about 8 to 10 days after inoculation. The dilutions of different samples varied according to the rapidity with which their strength diminished, but it was customary to prepare 1: 1,000, 1: 100, 1: 50, 1: 25, and 1: 10 dilutions, and undiluted stock suspension. The testing of different samples after the preliminary titration was, of course, much simplified, as in a subsequent titration fewer dilutions were sufficient to estimate the strength of the virus. Strict asepsis was exercised in handling the vaccine throughout the experiments.

For comparison the regular skin vaccines were employed. These had to be put up as emulsions containing 40 to 50 per cent glycerol or 0.5 per cent phenol.

Virulence and Affinities of the Testicular Vaccine Virus.

The first point to determine was whether a strain of vaccine virus which had been propagated for successive generations during a

period of 3 years would acquire an ascending virulence for the testicular tissues while suffering a gradual loss of its affinity for the tegumentary system. In the beginning the strain which was passed on to the testicular tissue showed a lower virulence for this organ, but it also showed a correspondingly low virulence for the dermal tissue. Upon attaining a certain degree of virulence for the testes the virus manifested a parallel increase of activity for the skin, showing no disproportion between the titers for the two kinds of tissue. It may be assumed, then, that by a prolonged passage through the testes, the affinity for the skin has in no wise been diminished. The maximum titer obtained by a testicular product in rabbits was that which produced a confluent eruption on the skin of rabbits in a dilution of 1: 10,000. Such specimens were not frequently obtained, and the result probably depends upon the suitability of the animal used. There are considerable individual variations in the susceptibility of the rabbit and the calf, and it is not rare to get a specimen that requires a 1: 100 dilution in order to produce a confluent reaction. The titers with the rabbits averaged about 1: 1,000. Of course, a specimen possessing the activity to produce a confluent reaction in a 1 : 100 dilution is already strong enough to insure 100 per cent of takes among primates. The individual variations in suitability for producing a testicular virus are paralleled by the susceptibility of the skin of the same animal to the vaccine virus, regardless of the mode of propagation.

It has been stated elsewhere[1] that a highly potential testicular vaccine can easily set up vaccinia orchitis in the rabbit in dilutions as high as 1: 100,000, while on the skin the same dilution produces a few eruptions. In this respect the virus seemed to have acquired an increase in virulence for the testicle but not necessarily to have lost its dermatophilic property. It still remained to be seen whether this orchitophilic adaptation of the virus was associated also with the general increase of virulence for other internal organs and tissues. In order to determine this point we tested the emulsions of lungs, liver,. spleen, kidneys, and lymph glands, removed 5 days after inoculation, of the rabbits which had been successfully inoculated intratesticularly with the testicular virus. As controls a number of rabbits were intratesticularly inoculated with unadapted virus,

which, however, caused a marked orchitis. In a second series of animals the testicular strain was used on the skin, causing the latter to produce a confluent eruption, while several animals were vaccinated with the regular skin strain to serve as controls. The results were uniformly negative, except for a few eruptions in the areas inoculated with the lymph nodes in a few instances where the virus, irrespective of whether it was of testicular or dermal origin, was given intratesticularly.

Localization of the Vaccine Virus after Subcutaneous and Intravenous Inoculations.

The introduction of small quantities of the testicular virus, such as 1 cc. of a 1: 1,000, 1: 10,000, etc., dilution, into the blood circulation or under the skin of the rabbit produced no appreciable local or general symptoms. The injection of 1 cc. of a 1: 100 dilution, however, sometimes caused a local inflammation and rise in temperature on the 4th and 5th days. No general eruption was ever observed. The injection of 1 cc. of a 1: 10 dilution was sometimes accompanied by a high temperature for 3 days, and, in the case of subcutaneous injection, local tumefaction and edema, but no generalized eruption. The injection of 1 cc. or more of the undiluted emulsion produced a grave illness of 3 or 4 days, with a fever lasting for 3 days. Generalized eruptions, particularly numerous on the mucous membranes of the nose, lips, mouth, and genitalia, were observed. Camus[2,3,4] noted a similar phenomenon with the skin vaccine.

The examinations of different tissues removed from the animals showed that in cases of intravenous inoculation of 1: 10 and 1: 100 dilutions, the lymph glands and sometimes, but seldom, one of the testes contained some virus, but no bilateral orchitis or marked reaction was obtained. In case of a higher dilution than 1: 1,000 we occasionally demonstrated a small quantity of the virus in the lymph

[2] Camus, L., De la vaccine généralisée expérimentale. Conditions de sa production, *Bull. Acad. méd.*, 1916, lxxvi, 342.

[3] Camus, L., Réproduction de la vaccine généralisée chez le chien, *Bull. Acad. méd.*, 1916, lxxvi, 376.

[4] Camus, L., La vaccine généralisée expérimentale chez la génisse et chez le singe, *Bull. Acad. méd.*, 1916, lxxvi, 433.

nodes, but never in the testes or other organs. Even the injection of 1 cc. of the undiluted vaccine failed in two experiments to localize in the testes. The other organs, except the lymph glands, were negative.

The corresponding series of experiments with subcutaneous inoculation did not bring about generalized eruptions even with the strongest dose used. A small amount of the virus in the adjacent lymph nodes was occasionally demonstrated, but far less frequently than in the intravenous series.

The rabbits which received the intravenous and subcutaneous inoculations of the virus were tested for their susceptibility to a subsequent application of a powerful vaccine, both the testicular and the dermal, within several weeks. They were found to be refractory to the new vaccination, although some of them had originally received only 1 cc. of a 1: 10,000 dilution. The testes of these rabbits were also insusceptible to the inoculation with a highly active virus. The immunity brought about by the intravenous or subcutaneous inoculation of the virus was altogether comparable with that conferred by a regular procedure on the skin. A detailed report of this phase of the work will be made later.

Viability and Resistance of the Testicular Vaccine Virus.

Data concerning the viability and resistance of vaccine virus ought to be abundant, but one does not easily find details of a systematic investigation. From the time of Jenner the resistance of the virus to desiccation has been known, and it was proved nearly 50 years ago[5] that it resists the action of glycerol when the latter is used in the proper concentration. Later Umeno[6] and others found that phenol in a concentration below 1 per cent does not perceptibly decrease its virulence. Yet much of what was done years ago seems to have been overlooked, and it may be of sufficient interest to publish here the experimental data obtained by the writer during the past 3

[5] Copeman, S. M., Vaccination. Its natural history and pathology, London, 1899, 156.

[6] Personal communication from Professor S. Umeno, Director of the Imperial Institute for the Preparation of Vaccine Virus, Tokyo, Japan.

years. Naturally, the chief object of the experiments was to study the testicular vaccine virus, but in some instances, controls with the regular vaccine virus were made as far as it was possible. From the nature of the latter, however, no experiments could have been carried out to test its viability in a suspension without antiseptics such as glycerol or phenol, while it was possible to do so with the bacteria-free testicular virus.

Survival of the Vaccine Virus in Distilled Water and Glycerolated Media, at Different Temperatures.

April 2, 1915. Three sets of eight test-tubes each were used. After sterilization, 11.6 cc. of distilled water were added to the first tube of each set, and to the other seven tubes 11.6 cc. of 10, 20, 40, 50, 60, 80 per cent, and pure glycerol respectively. The contents of each tube were mixed with 0.4 cc. of the stock emulsion of the testicular strain, No. 948 emulsion, which had the titer of 1: 1,000 (confluent). One set was placed in a refrigerator at 4°C., the second set at 18°, and the last at 37°C. 0.5 cc. was taken from each tube and tested as usual on the skin of rabbits, three or four rabbits being used in order to test the three sets (24 tubes) simultaneously. At first tests were made daily, or every other day, but later at semimonthly, monthly, or longer intervals. Tables I, II, and III give the results.

The most striking point demonstrated in the foregoing experiments is that the vaccine virus retains its virulence much longer in distilled water than in any of the glycerolated media. Pure glycerol destroyed the virus to a considerable extent in a week and completely within a month, even at a temperature of 4°C., while the virus in water remained very active after 1 year. As the concentration of glycerol diminished its destructive effect was less noticeable, and in 10 to 20 to 40 per cent the virus remained active for at least 6 months. At 18°C., the temperature of our laboratory, the virus deteriorated rapidly. The virus was killed in 5 days in pure glycerol, in 1 month in 60 to 80 per cent, in 2 months in 10 to 20 per cent glycerol, while it was still very active in water after 3 months. These differences are much more accentuated at 37°C., where the virus was no longer alive in pure glycerol after 24 hours. In 80 per cent glycerol it was avirulent in 6 days, in 60 per cent in 7 days, in 50 per cent in 9 days, and in 10 to 20 to 40 per cent in 28 days. On the other hand, in water

TABLE I.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 4°C.

April 9, 1915.

Medium.	Days.																
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271	360
Water										Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++
10 per cent glycerol										"	"	"	"	"	"	+	<+
20 " " "										"	"	"	"	"	"	<+	−
40 " " "										"	"	"	"	"	"	<+	−
50 " " "										"	"	"	"	++	++	<+	−
60 " " "										"	"	"	"	++	+	−	
80 " " "	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl	"	"	"	++	++	−		
100 " " "	"	"	"	"	"	"	+++	++	+	+	<+	−	−	−	−		

The reactions are designated as confluent (cfl.), almost confluent, +++, ++, +, <+, <<+, and finally, 1 to 3 eruptions to an area 10 by 20 cm., to which 0.5 cc. of each dilution was applied.

TABLE II.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 18°C.

April 9, 1915.

Medium.	Days.															
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271
Water										Cfl.	Cfl.	+++	+++	Cfl.	–	–
10 per cent glycerol										"	"	++	+	–	–	–
20 " " "										"	"	++	+	–	–	
40 " " "										"	"	+	<+	–		
50 " " "									Cfl.	"	+	<+	–	–		
60 " " "				Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	"	"	+	–	–	–		
80 " " "	Cfl.	Cfl.	Cfl.	"	+++	+++	+++	++	++	++	<+	–	–	–		
100 " " "	+++	++	+	<+	–	–	–	–	–	–	–					

TABLE III.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 37°C.

April 9, 1915.

Medium.	Days.													
	1	2	3	4	5	6	7	9	11	13	15	28	43	60
Water	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++	<+	<+	<+	<+	<<+
10 per cent glycerol	"	"	"	"	"	"	"	++	+	<+	<+	–	–	–
20 " " "	"	"	"	"	"	"	++	+	+	+	<+	–	–	–
40 " " "	"	"	"	"	"	+++	++	+	+	+	<+	–	–	–
50 " " "	"	"	"	+++	++	+	+	–	–	–	–			
60 " " "	"	"	"	+	+	<+	–							
80 " " "	++	+	+	<+	<+	–	–							
100 " " "	–	–	–	–	–									

a trace of the virus was still present for as long as 2 months, when it still showed a few eruptions on test. The powerful vaccinicidal property of glycerol is well brought out in this set, and in any experiment bearing upon the viability of the virus this factor should be considered. The persistence of the virus in water or in 10 to 20 per cent glycerolated water at the temperature of 37°C. is remarkable and becomes important in interpreting the results in cultivation experiments.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures.

May 24, 1915. Experiments were made similar to the foregoing but with distilled water, 0.9 per cent saline solution, Ringer's solution, 50 per cent glycerol, 0.5, 1, and 2 per cent aqueous, and 50 per cent glycerolated solutions of phenol as diluents. To 19.6 cc. of each of the solutions was added 0.4 cc. of testicular virus, No. 992 emulsion. The three sets of tubes were placed at 4°, 18°, and 37°C. respectively. The results of the tests are given in Table IV.

Table IV confirms the findings of the preceding series of experiments and further shows that at 4°C. the virus was best preserved in Ringer's solution and in 0.5 and 1 per cent phenol water, being still active at the end of 1 year. In distilled water it was weaker than in saline solution, the latter being almost as good a medium as Ringer's solution. The deteriorating effect of 50 per cent glycerol was marked in this instance. The addition of phenol in 0.5 per cent did not affect the action, although 1 per cent phenol plus 50 per cent glycerol killed the virus more quickly than either of them separately. Phenol in a 2 per cent solution and agitation of the virus with an excess of chloroform for 3 hours destroyed the virus within 24 hours. The results at 18°C. and at 37°C. indicate that there is a more complete and rapid destruction of the virus with a rise of temperature. An interesting feature seems to be the longer, if not better survival of the virus in a carbolized solution (0.5 per cent water) than in Ringer's solution, saline solution, or distilled water. In the first 7 days, the activity of the virus in the latter solutions was greater than in the carbolized medium, however.

TABLE IV.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures. 19.6 Cc. of Diluent + 0.4 Cc. of No. 992 Emulsion (1: 50 Dilution).

May 24, 1915.

Temperature.	No. of days.	Water.	0.9 per cent saline solution.	Ringer's solution.	50 per cent glycerol.	Phenol in water.			Phenol in 50 per cent glycerol.			Shaken 3 hrs. in aqueous suspension with.	
						0.5 per cent.	1 per cent.	2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	Chloroform.	Ether.
°C.													
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	–	Cfl.	+++	–	–	+
	2	"	"	"	"	"	"	–	"	+++	–	–	+
	3	"	"	"	"	"	"	–	"	+++	–	–	+
	4	"	"	"	+++	"	"		"	+++		–	+
	5	"	"	"	+++	"	"		"	++		–	+
4	7	"	"	"	++	"	"		++	+			–
	17	"	"	"	+	"	"		+	<+			
	37	"	"	"	+	"	"		+	<+			
	226	+++	"	"	–	"	"		<+	<+			
	276	<+	+++	"	–	"	"		–	–			
	365	++	+++	"		"	"						
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	–	+++	+++	–	–	<+
	2	"	"	"	"	"	+++	–	+++	+++	–	–	<<+
	3	"	"	"	"	"	++	–	++	++	–	–	–
	4	"	"	"	"	"	++		++	+			–
	5	"	"	"	<+	"	+		<+	<+			
18	7	"	"	"	1 eruption.	"	+		–	–			
	17	+	"	+	–	+	<+		–	–			
	37	+	+	+	–	+	–		–				
	173	–	–	–	–	–	–		–				

37	1	Cfl.	Cfl.	Cfl.	Cfl.	+++	++	−	<+	<+	−	−	−
	2	"	"	"	+++	+++	+	−	++	<<+	−	−	−
	3	"	"	"	+	+++	+	−	<+	−	−	−	−
	4	"	"	"	+	++	+		(?)	−			
	5	"	"	"	<+	+++	<+		<+	−			
	7	++	++	+++	−	+++	−		−	−			
	17	<+	<+	−	−	<+	−		−				
	37	−	−	−	−	<<+							

Survival of the Vaccine Virus in Different Atmospheres.

In order to find whether a gradual deterioration of the vaccine virus could be delayed or prevented by preserving the virus in different kinds of gases, we placed the vaccine in sealed ampules containing either hydrogen, nitrogen, oxygen, carbon dioxide, or air. For this purpose the testicular vaccine, No. 1,035 emulsion (1:1,000 titer), was diluted 10 times with sterile distilled water, the gases were passed through the vaccine, and the receptacles hermetically sealed by fusing the drawn portions with flame. The vaccine was not exposed to heat.

The appearance of the vaccine was not altered by hydrogen, nitrogen, or air, but the passage of carbon dioxide caused a clearing of the diffuse turbidity, the precipitates adhering to the wall of the container, while with oxygen, granular precipitates appeared.

Three duplicate sets placed at 4°, 18°, and 37°C. respectively were used. The results obtained after a period of 21 days are shown in Table V.

TABLE V.

Survival of Vaccine Virus in Various Atmospheres.

Gas.	Results after 21 days.		
	4° C.	18° C.	37° C.
Hydrogen (sealed)	Cfl.	+++	–
Nitrogen "	"	++	–
Oxygen "	"	–	–
Carbon dioxide (sealed)	"	–	–
Air (sealed)	"	++	–
" (open tube)	"	+	–

The virus retained its virulence in all the gases for 3 weeks when kept at 4°C. but became avirulent at 37°C. From the results obtained at 18°C., however, it may be concluded that in sealed ampules containing hydrogen, nitrogen, or ordinary air, the virus retained its virulence somewhat better than in an open receptacle. Pure oxygen or carbon dioxide gas destroyed the virus completely at the same temperature.

Effect of Acid, Alkali, and Germicides upon the Vaccine Virus.

Knowledge concerning the resistance of the vaccine virus to various substances is a prerequisite for handling it properly. In a series of experiments hydrochloric acid, sodium hydroxide, tricresol, and phenol in varying concentrations were mixed with a testicular vaccine emulsion. Tests were made on rabbits at the end of 24 hours, 4 days, and 6 days respectively at 4°C. Table VI shows the results.

The table shows that the vaccine virus is completely destroyed by sodium hydroxide added in a concentration greater than 1: 200,

TABLE VI.

Effect of Acid, Alkali, and Germicides.

Substance.		Testicular vaccine (no bacteria). Tested for vaccine.			Fresh skin vaccine (some cocci).					
					Tested for vaccine.			Tested for bacteria.		
		24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.
	per cent									
Hydrochloric acid.	4	—	—	—	—	—	—	—		
	2	—	—	—	—	—	—	—		
	1	—	—	—	—	—	—	—		
	0.5	1 eruption.	—	—	A few eruptions.	—	—	—		
Sodium hydroxide.	4	—	—	—	—	—	—	—	—	
	2	—	—	—	—	—	—	—	—	
	1	—	—	—	—	—	—	+	—	
	0.5	—	—	—	—	—	—	+	—	
Tricresol.	2	—	—	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	—	—	—
	0.5	Cfl.	—	—	Cfl.	—	—	—	—	—
	0.2	"	Cfl.	Cfl.	"	Cfl.	Cfl.	+	+	+
Phenol.	2	—	—	—	—	—	—	—	—	—
	1	Cfl.	Cfl.	+++	Cfl.	Cfl.	+++	+	—	—
	0.5	"	"	Cfl.	"	"	+++	+	+	+
	0.2	"	"	"	"	"	+++	+	+	+
Control.		Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+	+	+

while hydrochloric acid destroyed it almost completely in a corresponding concentration. On the other hand, the contaminating micrococci of the fresh skin pulp showed a different relation, resisting the action of even 1 per cent sodium hydroxide solution for at least 24 hours, while they were completely sterilized by a 0.5 per cent hydrochloric acid solution. The action of tricresol and phenol is similar, the difference being quantitative rather than qualitative. The virus resisted 0.2 per cent tricresol or 0.5 per cent phenol for many days, as did also the contaminating bacteria. In the case of phenol, and in a lesser degree tricresol, the destructive effect was comparatively more severe upon the micrococci than upon the vaccine virus. The margin was, however, narrow. A bacterial spore cannot be sterilized by a concentration which will not destroy the vaccine virus completely.

The action of iodine was next studied in various ways, because of its effectiveness as a germicide. It was employed as a local antiseptic in the form of an alcoholic solution or as Lugol's solution. In a series of experiments we made a number of dilutions of tincture of iodine by using 10 per cent ethyl alcohol water as diluent. To 0.9 cc. of each dilution, 0.1 cc. of a 1: 10 dilution of a strong testicular vaccine virus was added, the mixture incubated for 1 hour at 37°C., and then tested on rabbits. It was found that the diluent alone, that is a 10 per cent alcohol water, exerted no effect. On the other hand, the mixtures containing a dilution of the tincture of iodine above 1: 10,000 became avirulent. The mixture which contained a dilution of 1: 100,000 gave several eruptions instead of the confluent reaction which took place when controls without iodine were used. The experiment demonstrates how destructive this element is for the vaccine virus. Lugol's solution (iodine 1 part, potassium iodide 2 parts, and water 300 parts) destroyed the virus in a 1: 100 dilution but had no effect in a 1: 1,000 dilution. Attempts were made to influence the course of the vaccine reaction by administering a considerable amount of Lugol's solution or sodium or potassium salts by intravenous or subcutaneous injections for several days before and after the vaccination. No effect was perceptible. The iodide salts failed to reduce the virulence of the vaccine virus even when mixed *in vitro* in a 30 per cent solution and kept 1 hour at 37°C.

Attempts were made to sterilize the vaccine virus simultaneously

with its application to the skin or at various intervals afterwards. It was found that tincture of iodine in a concentration stronger than 1: 10 inhibits the development of the eruption; in 1: 400 dilution, it prevented the process from being confluent. Lugol's solution used in full strength reduced but failed to check the infection. The application after 24 hours of tincture of iodine in concentrated forms did not noticeably influence the vaccine infection.

So far no visible organism has been found as the causative agent of vaccinia. From the viewpoint of classification it seemed important to study the effects of certain protoplasmic poisons on the virus. For this purpose 0.1 cc. of testicular vaccine, No. 1,062 emulsion, was mixed with 0.5 cc. of sodium oleate, sodium taurocholate, sodium glycocholate, and sodium cholate, in varying concentrations. After 1 hour at 37°C. the mixtures were tested on rabbits. It was found that all the salts destroyed the vaccine virus in a 1: 10 dilution. In a 1: 100 dilution a small portion of the virus still survived, while the virus in control tubes was capable of producing a confluent reaction.

Effect of Desiccation upon the Vaccine Virus.

Vaccine virus is known to withstand desiccation for a long time, but more exact knowledge is desired as to its reaction to dryness, the length of time it will survive in the dry state, and how the dried vaccine virus compares with moist emulsions at different temperatures. Many microorganisms, especially those which pass the filter, resist desiccation and remain viable for a long time. Most enzymes retain their activity better in the dry than in the moist state, especially at the higher temperatures. The question becomes one of considerable importance in the case of the vaccine virus, because of the rapid deterioration which attends the moist preparation of the virus in tropical countries. If the dried vaccine proved to resist the conditions of moisture and temperature similar to those of a tropical climate better than the liquid emulsion, it would be a great advantage to preserve the vaccine virus in the dried form.

On several occasions we have dried quantities of the organ paste of testicular vaccine virus by means of a vacuum pump. After desiccation quantities were weighed, powdered, and preserved in

hermetically sealed ampules or left in an open receptacle. One set of specimens was placed at 4°C., some at 18°, and others at 37°. The controls consisted of aqueous emulsions of the undried portion of the same tissue paste.

The reduction in weight through desiccation was not uniform, and no constancy could be expected on account of the variation in degree of edema of the organs (Table VII).

TABLE VII.

Reduction of the Weight of the Vaccine Paste through Desiccation.

Emulsion No.	Organ.	Original weight.	Residue.
		gm.	*gm.*
866	Testis.	2.0	0.38
876	"	2.5	0.38
877	"	2.0	0.3
878	"	2.0	0.32
1,045	"	1.85	0.32
1,046	"	1.6	0.25

The vaccine virus did not in any instance show its original titer after desiccation. The loss of virulence, as determined by employing corresponding quantities of the dried and moist materials, amounts to half or even more of its original strength. This was unexpected, but was true with all the dried materials. The insolubility which attends the desiccation of the vaccine paste may play a part in the loss of virulence. To resuspend and dissolve the powdered material is difficult. The results obtained during a period of 18 months indicate that the dried material remains still viable, although reduced to one one-hundredth or less of the original titers, for about 12 to 18 months at 4°C. and 18°C., but becomes inert within 30 to 60 days at 37°C. The control specimens in the moist state showed similar relations.

From the above findings it is evident that the process of desiccation as carried out is considerably destructive to the vaccine virus, and that it does not protect it from the gradual deterioration due to age which takes place at different temperatures.

SUMMARY.

1. The virulence of vaccine virus for the testicular tissues increases until its maximum is finally reached. The selective increase is not associated with any loss, reduction, or modification in its virulence for the skin. A highly potent testicular vaccine is also highly active for the skin.

2. The testicular strain of vaccine virus has no more tendency to localize in various organs than the ordinary skin strain. Both may localize in adjacent lymph nodes when introduced intravenously, subcutaneously, or intratesticularly in sufficiently large quantities, but other organs are not involved.

3. Intravenous inoculation of an excessive amount of a powerful vaccine virus (1 to 2 cc. of undiluted stock emulsion), irrespective of whether it is from the testis or the skin, will result in a generalized eruption over the entire body surface of rabbits. The eruption may be confluent on mucous membranes of the mouth, nostrils, genitalia, etc. Intratesticular or subcutaneous inoculations of the same virus fail to produce this effect.

4. Subcutaneous or intravenous introduction of much smaller quantities of the virus does not cause an appreciable local or general reaction in the rabbit. But the animals which have once received these injections become refractory to a subsequent vaccination as applied to the skin. It seems probable that an active immunity has been conferred.

5. Experiments on the viability and resistance of the testicular strain of vaccine virus indicate that the virus is best preserved when emulsified with Ringer's solution or 0.9 per cent saline solution. Distilled water, while apparently one of the best diluents, fails to keep the virus active as long as Ringer's or saline solutions. As would be expected, the lower the temperature is, the longer the virus retains its viability. At 18° or 37°C., the deterioration of the virus proceeds rapidly. However, a small part of the virus survives after many weeks' standing at 37°C.

6. Of the two most commonly employed chemical agents for the ripening (eliminating bacteria) process of the green vaccine pulp, glycerol and phenol, the latter is the less injurious. Phenol in con-

centration above 2 per cent destroys the virus within 24 hours at any temperature, but it has almost no injurious effect when used in 0.5 to 1 per cent. On the other hand, glycerol is a powerful vaccinicide. When used in full strength it destroys the virus within 24 hours, even at 4°C. In a concentration of 40 per cent, that ordinarily recommended for the ripening, the virus retains some of its virulence for about half a year at 4°C., while at higher temperatures the same concentration kills the virus within 1 to 2 months. The virus preserved in distilled water or Ringer's solution under similar temperature conditions remains more active during this period. From this it may be concluded that glycerol is not an indifferent agent, as is assumed by many, but a powerful vaccinicide when used in high concentrations. The injurious effect is markedly accelerated at 18° or 37°C.

7. The vaccine virus retains its virulence better in a sealed tube containing either hydrogen, nitrogen, or air than in an open receptacle. The virus deteriorates when placed in a sealed tube with oxygen or carbon dioxide.

8. Desiccation decreases to a considerable degree the virulence of the vaccine virus. In the dried state the virus retains its viability about as long as does the emulsion, but it is not protected from the deterioration caused by age under various conditions.

9. Iodine is a powerful disinfectant for the vaccine virus, but its sodium and potassium salts have no effect. Various bile salts destroy the vaccine virus when employed in sufficient concentration.

SURVIVAL OF POLIOMYELITIC VIRUS IN THE BRAIN OF THE RABBIT.

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The consensus of opinion among investigators in America and Europe is that the causative microorganism of poliomyelitis is a so called filter passer. The filterable organism or virus of poliomyelitis possesses definite properties through which it may be identified, and among these the most decisive is its ability to incite experimental poliomyelitis in the monkey. In common with other filter passers, the microbic cause of poliomyelitis is very minute. It has not been detected with certainty by microscopic examination in infectious filtered fluids. There are reasons for believing that in artificial cultures the microorganism gives rise to colonies visible to the naked eye and composed of masses of minute organisms which have been called globoid bodies.[1] Similar globoid bodies have been detected in microscopic preparations of the nervous organs[2,3] and once in the circulating blood of an infected monkey.[3]

Another point of view, emphasized more recently, is based on the studies of Mathers, Rosenow, Nuzum, and their associates.[4] According to these investigators, epidemic poliomyelitis is caused by a polymorphous streptococcus, which induces paralysis and histological changes characteristic of the disease in rabbits as well as in monkeys.

[1] Flexner, S., and Noguchi, H., *J. Am. Med. Assn.*, 1913, lx, 362.

[2] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[3] Amoss, H. L., *J. Exp. Med.*, 1914, xix, 212.

[4] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

Since the filtered virus of poliomyelitis, while highly active against monkeys, is practically without pathogenic power for rabbits, a wide difference of experimental results needs to be explained in order to bring the two points of view into harmony.

Bull,[5] in this laboratory, attempted to confirm the work referred to with the polymorphous streptococcus, but without success. He carried out a large series of inoculations of streptococci derived from poliomyelitic human and monkey tissues without, in a single instance, either in rabbits or monkeys, inducing clinical symptoms or pathological lesions identifiable with those of epidemic poliomyelitis. Moreover, his efforts to immunize animals with the streptococcus so as to obtain a neutralizing serum for or to protect them against infection with the filtered virus, as Rosenow claims to have done, were wholly unsuccessful. More recently, Bull has again tried, unsuccessfully, to render a monkey immune to the virus by large intravenous injections of streptococci cultivated from the brain of a poliomyelitic human case. The protocols of this experiment follow. The question, therefore, arises as to the source as well as the significance of the streptococci found not infrequently in poliomyelitis. Smillie[6] found that when the cultures are made from monkeys moribund and slowly dying, or from animals which have been dead some hours, streptococci are frequently present, not only in the nervous organs, but even more abundantly in the abdominal viscera. In other words, the streptococci exhibit the characters of secondary, agonal invading microorganisms. The unreported experiment of Bull with streptococci follows. *Macacus rhesus* monkeys were used.

Monkey A.—Apr. 25, 1917. Injected intravenously the centrifuged sediment from a 24 hour growth in 60 cc. of ascitic dextrose broth of the third generation of streptococcus obtained from human poliomyelitic brain. The monkey remained active. Apr. 27. Injected intravenously the growth from 56 cc. of the same medium, third generation of the same streptococcus, and intracerebrally the growth from 14 cc. of the same culture. The monkey became irritable, but remained active, and was normal on May 1 when another intravenous injection from 60 cc. of the third generation culture was made. May 5. A fourth injection was given.

This monkey, the serum of which was shown on May 22 to agglutinate the strain of streptococcus in a dilution of 1:4,000, was tested (*a*) for neutralizing action on the filtered poliomyelitic virus, and (*b*) for protection against an intracerebral inoculation of the same virus.

Monkey B. Neutralization Test.—May 22. 2 cc. of serum from Monkey A were mixed with 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic monkey cord (active virus), incubated for 2 hours at 37°C., and placed

[5] Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557.

[6] Smillie, W. G., *J. Exp. Med.*, 1917, xxvii, 319.

over night at 4°C., then injected intracerebrally into Monkey B. May 30. Animal excited. June 1. Ataxic. June 2. Legs paralyzed; back weak. June 3. Prostrate. June 4. Died.

Autopsy.—Typical gross and microscopic lesions of poliomyelitis were present in the central nervous organs.

Protection Test of Monkey A.—May 22. After blood was withdrawn for the serum an intracerebral injection of 0.5 cc. of active virus was made. May 27. Monkey excited and ataxic. May 28. Prostrate. May 29. Died.

Autopsy.—Typical poliomyelitic lesions were present in the central nervous system.

EXPERIMENTAL.

In order to study further the relation of the filterable virus of poliomyelitis to the rabbit, with the special view of bringing out resemblances to or distinctions from the streptococcus and of determining its power of survival in the brain *in vivo*, inoculations of the virus were made into the brain of that animal. There was no expectation of inducing infection or of setting up paralysis. Bull[7] injected streptococci isolated from the human poliomyelitic brain intravenously into a rabbit without producing symptoms. The rabbit was etherized after 131 days. Streptococci corresponding antigenically with the strain originally injected were found to have survived in the brain. Bull also observed that local injections of streptococci from poliomyelitic tissue sometimes produce focal lesions in which the organisms survive for long periods. In other words, the polymorphous streptococcus is, under certain conditions, sufficiently adapted to the central nervous system of the rabbit to survive there, and sometimes sufficiently pathogenic to produce focal lesions in the meninges, cerebellum, medulla, cerebrum, and even in the spinal cord, and to thus induce clinical symptoms. The lesions do not, however, partake of the nature of the characteristic lesions of poliomyelitis.[5]

Hence, if a relation exists between the polymorphous streptococcus and the filterable virus, the latter might at least be expected to exhibit a fair degree of ability to survive in the brain of the rabbit. As the protocols which follow show, the period of survival is short.

[7] Unpublished experiment.

Rabbit A.—Nov. 21, 1916. Under ether anesthesia 0.5 cc. of a suspension of equal parts of active poliomyelitic monkey cord and isotonic salt solution was injected intracerebrally. The rabbit remained well. Dec. 14. The animal was etherized and the brain removed aseptically. There was no visible lesion at the site of inoculation. A 10 per cent suspension of the brain tissue from the region below the point of needle penetration through the skull was prepared for injection into a *Macacus rhesus* (Monkey C).

Monkey C.—Dec. 14, 1916. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit A, which had received the poliomyelitic virus 22 days previously. The monkey remained well.

Rabbit B.—Jan. 3, 1917. Injected heavy suspension of poliomyelitic virus according to the method already described. Jan. 16. Killed. There was no visible lesion at the site of inoculation. From the brain substance surrounding the site of inoculation, a 10 per cent suspension was prepared for injection into a *Macacus rhesus* (Monkey D).

Monkey D.—Jan. 16, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of brain at site of inoculation of Rabbit B, which had received poliomyelitic virus 12½ days previously. The monkey remained well.

Rabbit C.—Mar. 5, 1917. Injected intracerebrally heavy suspension of poliomyelitic virus according to the method already described. The rabbit showed no symptoms. Mar. 12. Killed. There was no visible lesion at the site of inoculation. The brain was removed aseptically and a 10 per cent suspension of the brain substance around the site of inoculation was prepared for injection into a *Macacus rhesus* (Monkey E).

Monkey E.—Mar. 12, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit C, which had received poliomyelitic virus 7 days previously. The monkey remained well.

Rabbit D.—Apr. 3, 1917. Injected intracerebrally a heavy suspension of active poliomyelitic monkey cord. The rabbit remained well. Apr. 7. Etherized and brain removed aseptically. No visible lesion at site of inoculation. A 10 per cent suspension was prepared from the brain tissue at the site of inoculation and injected intracerebrally into a *Macacus rhesus* (Monkey F).

Monkey F.—Apr. 7, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit D, which had received poliomyelitic virus 4 days previously. Apr. 13. Both legs paralyzed. Apr. 14. Prostrate. Apr. 15. Died.

Autopsy.—The central nervous organs showed macroscopic and microscopic lesions of poliomyelitis.

DISCUSSION AND SUMMARY.

Suspensions of the central nervous tissues of monkeys, containing the active filterable virus of poliomyelitis, may be injected into the

brain of rabbits without setting up symptoms, provided the volume of injection does not cause dangerous increased intracranial pressure.

Aside from the pressure effects which develop quickly, no other symptoms or pathological lesions are produced by the suspensions.

The active virus of poliomyelitis survives in the brain of rabbits for 4 days, as determined by tests in the monkey, into which the excised site of injection in the rabbit brain is reinoculated. It cannot be detected by this test after the expiration of 7 days.

The virus of poliomyelitis is unadapted to the rabbit, and neither induces lesions nor survives long in the central nervous organs of that animal. In this respect it differs from certain streptococci cultivated from poliomyelitic tissues.

A monkey immunized to streptococcus cultivated from human poliomyelitic nervous tissues yielded a serum which agglutinated the streptococcus in high dilution, but was without neutralizing action on the filtered virus; and the streptococcus-immune monkey was not protected against the effects of an intracerebral inoculation of the filtered virus.

The experiments recorded provide additional reasons for concluding that the streptococcus cultivated from cases of poliomyelitis differs essentially from the filterable virus and is not the microbic cause of epidemic poliomyelitis.

A STUDY OF THE NITROGEN METABOLISM AND OF ACIDOSIS AFTER THE TRANSPLANTATION OF A URETER INTO THE DUODENUM IN DOGS.

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Numerous reports, both clinical and experimental, have discussed the feasibility of implanting a ureter into some part of the intestinal canal, and have described methods of operative procedure. The literature was reviewed in 1909 by Steinke (1), who concluded that the results show that under certain conditions the ureter may be transplanted successfully. The failures, however, are numerous, the ultimate results uncertain, and a fatal termination is so apt to occur that no definite claim for the operation can as yet be made. Most experimental studies, as those recently made by Steinke (1909), Stewart (2) (1910), and Sweet and Stewart (3) (1914), have been especially concerned with the ascending infection of the kidney and the mechanical dilatation of the ureter following operation.

In order to avoid some of these difficulties—peritonitis, ascending infection of the kidneys, and stenosis of the ureteral orifice where it enters the intestine, with resulting hydroureter and hydronephrosis—Sweet and Stewart devised the following method of operation.

The right ureter of the dog is isolated and severed close to the bladder, and the free upper end passed through the lumen of the greater pancreatic duct into the intestine. This operation is made possible in the dog by the fact that this animal possesses at least two pancreatic ducts, both of which are separate from the bile duct. In Sweet and Stewart's series (3) eight dogs were used, and in some, nephrectomy of the left kidney was done at a later date. In none of the animals was there evidence of infection of the kidney, but in some, distinct evidence of obstruction was found, and in all, death resulted after varying intervals of time with symptoms suggestive of an intoxication.

In experiments such as those cited above an intoxication in the absence of infection suggests two possibilities: intoxication from (*a*) disturbance of kidney function or (*b*) absorption of urinary constituents draining into the intestine. It was with the hope of throwing

some light upon this intoxication that the present study was undertaken at the suggestion of Dr. Sweet.

EXPERIMENTAL.

Six dogs were used; in two a ureter-intestinal transplantation[1] was performed and four were used for various controls. The dogs were kept in metabolism cages and all were kept on the same dietary régime. The diet contained 0.4 gm. of nitrogen per kilo and 70 calories per kilo of body weight. It consisted of beef heart, lard, bread crumbs, sugar, a little salt (2 gm.), and sufficient bone ash to ensure well formed feces. The food was mixed with about 300 cc. of water. During each period of collection of blood and urine food was withheld. The animals were catheterized at the end of every 24 hours. After each catheterization the bladder was washed out and the wash water added to the catheterized urine and that which was voided naturally, and the whole made up to a definite volume.

The study of the urine included the determination of the total nitrogen, urea nitrogen, and ammonia nitrogen. For the determination of total nitrogen and urea nitrogen in the urine the new Nesslerization method of Folin and Denis (4) was used, and for ammonia the aeration method of Folin and Macallum (5). Blood was obtained by aseptic puncture of the vena jugularis and utilized for the study of the non-protein nitrogen, the urea nitrogen, and carbon dioxide content. In the examination of the blood the method of Folin and Denis (4) was used for the determination of non-protein nitrogen and urea nitrogen, and Van Slyke's method (6, 7) for the carbon dioxide content. Tests for ketones with Rothera's method (8) were made from time to time. All analyses were made in duplicate and sometimes in triplicate.

The operative procedure, as carried out by Dr. Sweet, consisted first, in transplantation of the right ureter into the duodenum and then, after several days, the removal of the left kidney. In all the experiments Period A refers to control observations before the first operation, Period B to observations after transplantation of ureter but before removal of the opposite kidney, and Period C to observa-

[1] All operations were done under ether anesthesia by Dr. J. E. Sweet.

tions after removal of the opposite kidney. During Period A animals were studied before any operative procedure for periods of 4 days. After the first operation several days were allowed for recovery from the acute effects of the operation, and another period of chemical study was undertaken (Period B). Under Period B1 are given the results obtained with animals after transplantation of the ureter, and under Period B2, as control, the results with animals in which the ureter was ligated and sectioned without removal of the kidney. After the opposite kidney was removed the chemical studies of the blood were continued until the dog died (Period C).

TABLE I.

Dog No.	Period.	No. of experiments.	Urine. Total nitrogen per day.	Urine. Urea nitrogen per day.	Urine. Ammonia nitrogen per day.	Blood. Non-protein nitrogen per 100 cc. of blood.	Blood. Urea nitrogen per 100 cc. of blood.	Blood. Carbon dioxide content of plasma.	Remarks.
			gm.	*gm.*	*gm.*	*mg.*	*mg.*	*vol. per cent*	
1, 2, 3, 4	A	4	4.12	3.15	0.15	29.5	10.1	56	Control observations in normal animals.
1, 5	B1	2	4.59	3.47	0.22	38.3	14.3	56	Right ureter in duodenum.
6	B2	1	6.18	4.44	0.18	30.3	11.1	52	" " ligated.
1, 5	C	2				201.8	102.2	41	Left kidney removed after Period B1.

It is obvious that in Period C the chemical examinations were necessarily limited to the blood.

In two other dogs as a control the right ureter was cut and ligated, but as one developed distemper following the operation the results on only one are given (Table I, Period B2).

The general results are shown in Table I which gives the average results for all animals during each of the three periods. In Tables II and III the results in Periods B and C are given in detail in contrast to the averages of Period A.

A comparison of Periods A and B1 shows that in the latter there is a moderate increase in the total nitrogen, urea nitrogen, and am-

TABLE II.

Dog 5.

Period.	Date.	No. of observations.	Weight.	Urine. Total nitrogen per day.	Urine. Urea nitrogen per day.	Urine. Ammonia nitrogen per day.	Blood. Date.	Blood. No. of observations.	Blood. Non-protein nitrogen per 100 cc. of blood.	Blood. Urea nitrogen per 100 cc. of blood.	Blood. Carbon dioxide content of plasma.	Remarks.
	1916		*kg.*	*gm.*	*gm.*	*gm.*	*1916*		*mg.*	*mg.*	*vol. per cent*	
A Average.......	Oct. 9–15	6	16.1	4.86	3.09	0.20	Oct. 13–19	5	33.4	11.2	57	Normal.
B	Oct. 24			6.93	4.47	0.35	Oct. 24		39.2	10.4	52	Oct. 19. Right ureter in duodenum.
	" 25		15.5	6.21	4.61	0.25						
	" 26	7	14.6	5.14	4.41	0.16	Oct. 26	4	42.8	18.2	48	
	" 27		14.5	6.43	4.29	0.29						
	" 28		14.3	5.48	4.61	0.41	Oct. 28		45.2	16.9	48	
	" 29		14.0	6.42	4.39	0.37						
	" 30		13.4	4.74	3.85	0.30	Oct. 30		50.0	23.4	59	
Average.......			14.4	5.91	4.38	0.31			44.3	17.2	51	Oct. 31. Left kidney removed.
C			13.8				Nov. 2		108.5	51.2	42	
			13.6				" 4		207.5	88.6	29	
			13.3				" 6	5	231.5	92.1	37	
			13.1				" 8		245.0	106.2	40	
			12.8				" 10		306.0	187.5	35	10 a.m. Died.

At autopsy slight hydronephrosis, cortex not greatly diminished; ureter not dilated but fluid could be forced into intestine only on pressure. Microscopically the kidney does not show evidence of infection.

monia of the urine and in the non-protein nitrogen and urea nitrogen of the blood. Whether this is due to retention of nitrogenous substances held back by injury to the kidney in consequence of the anastomosis, or to absorption of urine from the intestine, is a question which we shall discuss later. The change is relatively slight as compared with that of Period C in which a true nitrogen retention undoubtedly exists. In Dog 1 the probability of absorption of urinary constituents from the intestine cannot be entertained, for the amount of functioning kidney remaining was very slight and urine could be forced through the ureter only with great difficulty. Here, atrophy of the kidney and obstruction to the ureter, the opposite kidney having been removed, led to renal insufficiency and nitrogen retention. In Dog 5 the atrophy of the kidney was not so great, and ureteral obstruction doubtful; though this experiment is not so conclusive, it supports the view that the intoxication is due to retention and not to absorption from the intestine. The phenomena of Period C may be dismissed, therefore, as those of renal insufficiency and analogous to the changes observed by Karsner, Bunker, and Grabfield (9) after removal of both kidneys at consecutive operations. The changes in Period C in the carbon dioxide content of the plasma are noteworthy. A comparison with the findings in Period A, the control period, on an identical régime as regards food intake, indicates that the acidosis is due to change in kidney function. The search for ketones in these animals was negative. That all the changes we have found in the blood may arise after removing one kidney and ligating the opposite ureter is shown in Table IV.

The observations in Period B1 are difficult to explain. An increased output of urea and ammonia nitrogen in the urine and at the same time a retention in the blood cannot be connected with either disturbed elimination or absorption of urine from the intestine. Control observations on this point were unsatisfactory. In the animal in which, as control, one ureter was ligated without removal of the corresponding kidney (Period B2), the urinary output of nitrogenous bodies increased greatly but without appreciable change in those of the blood (Table I). Also, when in Period B2, of the experiment described here, urea is given by mouth, the nitrogen of both urine and blood is increased. These experiments, since they throw no

TABLE III.

Dog 1.

Period.	Date.	No. of observations	Weight.	Urine. Total nitrogen per day.	Urine. Urea nitrogen per day.	Urine. Ammonia nitrogen per day.	Blood. Date.	Blood. No. of observations.	Blood. Non-protein nitrogen per 100 cc. of blood.	Blood. Urea nitrogen per 100 cc. of blood.	Blood. Carbon dioxide content of plasma	Remarks.
	1916		*kg.*	*gm.*	*gm.*	*gm.*	*1916*		*mg.*	*mg.*	*vol. per cent*	
A Average.......	Nov. 13–16	4	15.2	3.63	2.99	0.15	Nov. 13–16	4	29.9	10.9	60	Normal.
B	Nov. 22	4	14.2	3.94	3.33	0.19	Nov. 22	4	32.6	18.8	59	Nov. 20. Right ureter in duodenum.
	" 23		13.9	5.36	3.94	0.19	" 23		32.5	15.2	62	
	" 24		13.9	5.27	3.78	0.18	" 24		33.4	15.2	61	
	" 25		13.7	4.84	3.36	0.16	" 25		34.2	14.7	64	
Average.......			13.9	4.85	3.60	0.18			33.2	16.0	61	
B (repeated).	Dec. 1	4	14.2	3.31	2.98	0.23	Dec. 1	4	29.6	9.9	58	
	" 2		13.9	3.29	2.27	0.22	" 2		30.2	10.4	56	
	" 3		13.7	2.78	1.26	0.17	" 3		34.1	8.0	58	
	" 4		13.5	2.73	2.15	0.15	" 4		35.2	10.3	56	
Average.......			13.8	3.03	2.16	0.19			32.3	9.9	57	

C			13.2				Dec. 9	3	81.2	60.8	50	Dec. 7. Left kidney removed.
			12.8				" 11		215.0	96.3	46	
			12.4				" 13		257.0	144.0	44	Died over night.

At autopsy marked hydronephrosis and hydroureter, only a thin rim of kidney cortex remaining. Fluid could be forced through the ureter into the intestine only on considerable pressure. Microscopically the kidney does not show evidence of infection.

light on the problem, are not reproduced in detail. Probably both after transplantation of the ureter (Period B1) and after its ligation and section without removal of the kidney (Period B2) there arises from some unknown cause an increased tissue catabolism accounting for the increased blood urea and non-protein blood nitrogen in Period B1 and for the increased nitrogenous excretion in the urine in Period B2. It is evident, however, that the explanation of the observations in Period B1 must await more detailed and more carefully controlled studies in which, among other things, the duodenal contents must be studied as to their content of nitrogen or other urinary constituents, both in normal dogs and those with anastomosis, in order to determine whether urine enters the intestine in any amount. These observations have been made in the present study, but are not satisfactory on account of the great difficulties of control.

TABLE IV.

Dog 6.

Date.	Blood.		
	Non-protein nitrogen per 100 cc. of blood.	Urea nitrogen per 100 cc. of blood.	Carbon dioxide content of plasma.
1916	*mg.*	*mg.*	*vol. per cent*
Nov. 18	34.8	10.4	52
" 19	29.3	9.4	53
" 20	28.2	10.2	55
" 21	26.6	8.3	52
Nov. 24	Left kidney removed; right ureter severed between ligatures; right kidney not removed.		
Nov. 25	53.4	17.1	45
" 26	79.6	32.8	43
" 27	122.0	44.6	44
" 28	154.0	105.0	44
" 29	198.0	113.0	43

The marked accumulation of non-protein and urea nitrogen in the blood in Period C shows that the transplantation of the right ureter into the duodenum had so interfered with the function of the right kidney as to render it inadequate for maintaining renal function after removal of the left.

SUMMARY.

1. The present work was undertaken to study the metabolism in the dog after a ureter-intestinal transplantation. Four dogs, Nos. 1, 2, 5, and 7, were originally operated upon. Two, Nos. 2 and 7, showed kidney infection; the other two were not infected, and in these the metabolism was studied; one of the latter (No. 1) showed a marked hydronephrosis and hydroureter.

2. Both after the transplantation of the right ureter into the intestine and the ligation of the right ureter, there is generally a moderately increased output of nitrogen in the urine and, in the former instance especially, a retention of nitrogen in the blood, but no change in carbon dioxide content in the blood. The significance of this is probably an increased tissue catabolism, the cause of which is doubtful without further work.

3. After removal of the left kidney subsequent to transplantation of the right ureter into the duodenum, renal insufficiency and resulting retention developed. The non-protein and urea nitrogen in the blood steadily increased and the carbon dioxide content of the blood diminished to the level characteristic of a moderate acidosis. No ketones were found in the blood The dogs died 5 to 10 days after the nephrectomy under conditions characteristic of suspended renal activity—deep respiration, unconsciousness, and sopor.

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METHOD FOR INTRAVENOUS INJECTION OF GUINEA PIGS.

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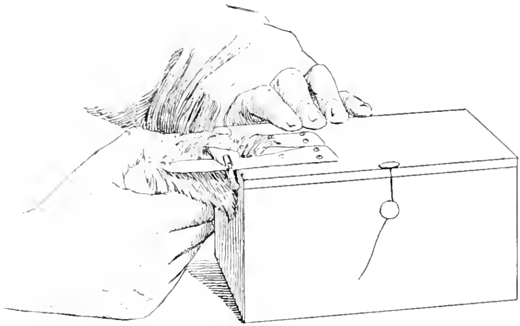
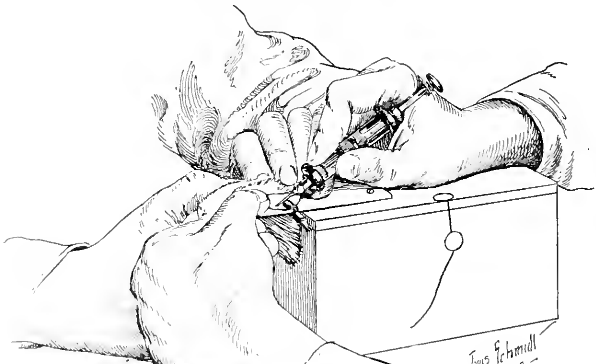
A great drawback to guinea pigs as experimental material lies in the difficulty of intravascular injections. Direct cardiac puncture is, of course, readily performed, but much of an injected fluid may escape into the pericardial sac, or the pleural cavity, and the operator be none the wiser. Dosage is well controlled when the jugular vein is used, but this must be bared by incision, and day to day injections are well nigh impossible. To meet the requirements of such work a technique has been devised whereby repeated injections may be made into an ear vein.

Most guinea pigs have relatively large ear veins, and those near the margin are extemely superficial. They lie, indeed, so close to the surface that in the attempt to get into them with a needle the vessel is usually transfixed or so torn that hemorrhage obscures the field. A greater obstacle to the injections is encountered in immobilizing the animal. To accomplish this, the guinea pig is placed in a small wooden box with a U-shaped opening in one end through which the head is thrust (Text-fig. 1). Extra space in the box is packed with towelling, and the lid brought down and secured, with a flexible wire, as closely as the respiration will permit. Fixed firmly to the lid are two thin plates of bone which project horizontally above and to either side of the guinea pig's head. These are platforms to which the ears may be fixed during injection. When needle and syringe are ready, the head is pressed firmly up against the platforms by an assistant, and one of the ears is drawn over and fastened lightly with a rubber-covered spring clamp (Text-fig. 1). Then the injection is made, away from the clamp, into a marginal vein (Text-figs. 2 and 3).



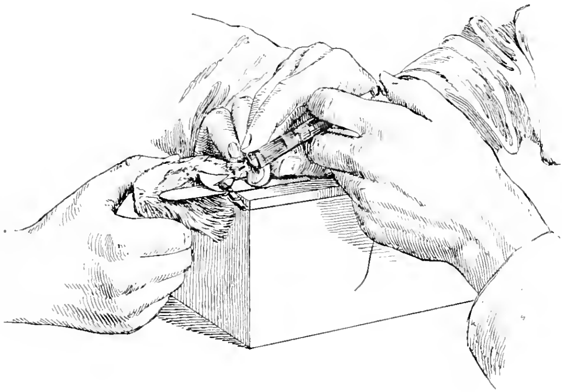
Text-Fig. 1. The animal's head is pressed firmly up against the platforms, to one of which the ear is fixed with a rubber-covered clamp.



Text-Fig. 2. With the thumb the ear is held flat and the vein distended. The bevelled side of the needle point lies against the vein as the latter is punctured.

In Text-figs. 2 and 3, the needle represented is of much larger caliber than that actually employed.

Some guinea pigs have small, indurated ears, the veins of which are not readily seen. These are unsuitable for use. Animals with black ears are a somewhat difficult material. In any instance a marginal vein should be chosen for the injection. The marginal vessels are not merely to be preferred: they are the only ones that can be used satisfactorily. The central veins, while larger, lie in a loose tissue which makes them difficult to pierce and renders leakage almost



TEXT-FIG. 3. The vein has merely been punctured, not entered further. Injection proceeds.

unavoidable. When the veins at the margin are used, any leak is at once evident: while the perivascular tissue is ordinarily so tough, that after a puncture either the injection proceeds successfully or no fluid leaves the syringe.

The needles employed should be fine (about No. 27 gauge, Brown and Sharpe), rather flexible, and at least half an inch long. Shorter ones are ordinarily so rigid as to be readily forced from the vein by any slight movement. It is important that the needle point should be bevelled obtusely. When an injection is given, the ear is held flat for the moment with the operator's thumb which by pressure distends the vein (Text-fig. 2). The needle is turned so that the

opening in it faces downward against the vessel as this is pierced. The merest puncture is sufficient. There should be no attempt to introduce the needle into the vein's lumen. All that is necessary is for an opening to be made in the vessel which will come opposite the opening in the needle. The tough perivascular tissue prevents any escape of material to either side. If the technique has been good, yet fluid fails to enter the circulation, the occurrence or absence of bleeding when the needle is withdrawn will show whether the vein has been punctured. If there is bleeding the needle may be thrust again into the little puncture wound, and now injection will often be successful. When large quantities of fluid are to be injected, the same puncture may be entered repeatedly.

The syringe should be absolutely tight and should contain no air, for the reason that successful puncture is most readily told by the slight yielding of the piston as the fluid begins to enter the vein; and this sign will be lacking when there is air in the barrel.

The technique has been described at length because strict adherence to it means success instead of failure. When it is rightly applied, injections up to 4 cc. are not difficult and may be accurately given day after day. The work is much less difficult than the injection of mice into the caudal vein, which is now so commonly practised.

A STUDY OF THE ANTISEPTIC PROPERTIES OF CERTAIN ORGANIC COMPOUNDS.*

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Interest in the selective behavior of dyes dates back to Ehrlich's work with methylene blue. However, Stilling was probably the first to call attention to the antiseptic action of dyes on bacteria. He examined the inhibitive effect of diphenyl- and triphenylmethane dyes and recommended auramine and methyl violet as good antiseptic agents. Penzoldt tested a series of dyes and found that methyl violet, malachite green, etc., were good inhibitors. Von Drigalski and Conradi carried the idea a step further and showed that some aniline dyes inhibited *B. typhosus* less than other bacteria present in feces and recommended the use of crystal violet for the isolation of *B. typhosus* from stools, etc. Loeffler, and later Conradi, studied this phase of the problem more thoroughly, the latter testing about 400 dyes, but since they limited their observations to the action of these substances on *B. typhosus*, they contributed nothing new concerning the specific behavior of the dyes. Churchman, however, developed these observations and showed that certain violet dyes (gentian violet, crystal violet, dahlia) were more inhibitive for Gram-positive than for Gram-negative bacteria. These findings were confirmed and extended by Krumwiede and Pratt, who studied a larger series of this group of dyes.

It is evident from this brief review that the triphenylmethane dyes constitute a group of substances toxic to bacteria and reacting in a partially specific manner in the sense of Bechhold and Ehrlich. But, owing to the fact that the investigators were more absorbed in the practical application of this property, little is known concerning the nature of the action. Since my problem was similar to theirs, and since their extensive investigations resulted in only a partially successful solution, it seemed that a more effective attack might be made possible by a better understanding of the factors concerned in the specific affinity manifested by these dyes.

The noteworthy fact gathered from the literature is that all the dyes used in the isolation of *Bacillus typhosus* (crystal violet, malachite

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green, brilliant green), as well as those studied by Churchman (gentian violet, crystal violet, dahlia), belong to the triphenylmethane group. Diamino or triamino triphenylcarbinol may be considered the basis of these dyes. Upon treatment with acid, these dye bases are converted into dyes themselves, the conversion, as is commonly accepted, being accompanied by a rearrangement to the quinoid form. A series of homologous dyes may be produced by substituting the hydrogen in the NH_2 group by alkyl or aryl radicals. This class of substances promised, therefore, to be a good starting-point for the study of the structural chemical factors involved in the action of dyes on bacteria. A series of representative compounds was selected and their action on a number of typical bacteria studied quantitatively under carefully controlled conditions.[1] It soon became apparent that it would be desirable to extend the list and include for comparison a number of the simpler aromatic amino compounds. Consequently, aniline, toluidine, and some of their alkyl derivatives, and a few other related compounds were tested.[2]

Technique.

Substances Used.—A list of the substances used and their chemical constitution are given in Table I. The compounds are arranged as nearly as possible in the order of their complexity. The list is by no means exhaustive. Other compounds might have been included but were not easily obtainable, while still others were ruled out because of their insolubility.

Method.—The details of the method used in testing the antiseptic property of a given compound are highly important. Although comparable and fairly constant results may be obtained under identical conditions, a variation in one or another of the factors involved will cause decided fluctuations in the results. The important factors to be controlled are the composition and reaction of the medium and the condition of the culture used in the test.

[1] On account of the war, only a limited number of the compounds selected could be obtained.

[2] The dyes used were all Grübler's and were presumably fairly pure. The auramine, as well as the aniline, toluidine, and other compounds tested, was kindly furnished by Dr. W. A. Jacobs of The Rockefeller Institute for Medical Research, and were obtained from either Kahlbaum or Schuchardt.

TABLE I.

List of Compounds Used.

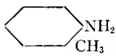
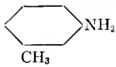
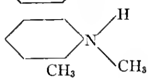
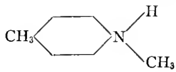
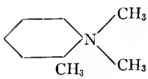
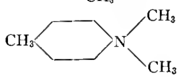
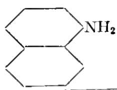
No.	Name.	Formula.	
1	Methylamine (mono-).	$NH_2.CH_3.HCl$	
2	" (di-).	$NH(CH_3)_2.HCl$	
3	" (tri-).	$N(CH_3)_3.HCl$	
4	Methyl alcohol.	$CH_3.OH$	
5	Ethyl "	$CH_3.CH_2.OH$	
6	Ethylamine.	$NH_2.C_2H_5$	
7	Diethylamine.	$NH(C_2H_5)_2$	
8	Aniline.	$C_6H_5.NH_2$	
9	Methyl aniline.	$C_6H_5.NH.CH_3$	
10	Dimethyl "	$C_6H_5.N(CH_3)_2$	
11	Ethyl "	$C_6H_5.NH.C_2H_5$	
12	Diethyl "	$C_6H_5.N(C_2H_5)_2$	
13	*o*-Toluidine.	$C_6H_4.CH_3.NH_2$	
14	*m*- "	$C_6H_4.CH_3.NH_2$	
15	*p*- "	$C_6H_4.CH_3.NH_2$	
16	N-Methyl *o*-toluidine.	$C_6H_4.CH_3.NH.CH_3$	
17	N- " *p*- "	$C_6H_4.CH_3.NH.CH_3$	
18	N-Dimethyl *o*- "	$C_6H_4.CH_3.N(CH_3)_2$	
19	N- " *p*- "	$C_6H_4.CH_3.N(CH_3)_2$	
20	α-Naphthylamine.	$C_{10}H_6.NH_2$	

TABLE I—*Continued.*

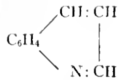
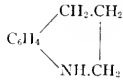
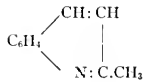
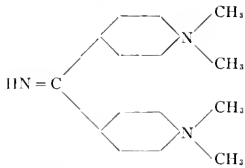
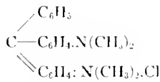
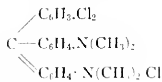
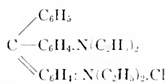
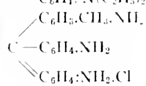
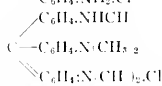
No.	Name.	Formula.
21	Quinoline.	
22	Tetrahydroquinoline.	
23	Quinaldine.	
24	Auramine.	
25	Malachite green.	
26	Victoria "	
27	Brilliant "	
28	Fuchsin (acid).	
29	Methylviolett B.	

TABLE I—*Concluded.*

No.	Name.	Formula.
30	Crystal violet.	$C_6H_4.N(CH_3)_2$ $C—C_6H_4.N(CH_3)_2$ $C_6H_4{:}\ N(CH_3)_2.Cl$
31	Gentian "	Mixture of crystal and methyl violet plus dextrin.
32	Methyl green.	$C_6H_4.N(CH_3)_2.CH_3Cl$ $C\quad C_6H_4.N(CH_3)_2$ $C_6H_4{:}\ N(CH_3)_2.Cl$
33	Aniline blue.	$C_6H_3.CH_3.NH.C_6H_4$ $C—C_6H_4.NH.C_6H_5$ $C_6H_4{:}\ NH.C_6H_5.Cl$
34	Dahlia.	$C_6H_3.CH_3.NH.C_2H_5$ $C—C_6H_4.NH.C_2H_5$ $C_6H_4{:}\ NH.C_2H_5.Cl$

Composition of the Medium.—That the presence of different amounts of colloidal or organic matter influences the action of dyes is well known. This is equally true of other antiseptics. An idea of the effect of different media on the antiseptic action of chemicals is obtained from the data shown in Table II. In these tests the only variable was the composition of the test media; a special peptone broth and standard beef extract broth were used. It is clear from the results that although the characteristic action of the drug is not modified by the medium, the effective concentration is appreciably altered. It is important, therefore, in testing a large number of drugs, to use a medium subject to as little variation in composition as possible. The following medium proved satisfactory:[3]

	per cent
Peptone	1.0
Potassium phosphate (dibasic)	0.5
Sodium chloride	0.5
Glucose	0.1

[3] Fairchild's peptone was used throughout the investigation. The salts and sugar were chemically pure.

TABLE II.

Action of Antiseptics in Peptone and Nutrient Broth Respectively.

Test substance.	Dilution.	Test media.	Test cultures.* 2	6	17	12	14
Dibromo-β-naphthol.	12,500	Nutrient broth.	–†	–	–	+++	+++
	25,000	" "	+	–	++	+++	+++
		Peptone "	–	–	–	+++	+++
	40,000	" "	+	+	++	+++	+++
Hexamethylenetetramine quaternary salt of p-chloroacetylaminotetraethyl p′, p″-diaminotriphenylmethane.	15,000	Nutrient broth.	+	+	+	++	+++
		Peptone "	–	–	–	+	+
	30,000	Nutrient "	+	+	+	+++	+++
		Peptone "	–	–	–	+	++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	10,000	Nutrient broth.	–	–	–	+	–
	20,000	" "	+	±	±	+++	++
		Peptone "	–	–	±	++	–
	40,000	" "	+	+	+	+++	++

* The numbers of the cultures correspond with those given in Table IV.

† – indicates no growth; ±, +, ++, +++ indicate the relative amount of turbidity at the end of 24 hours.

This culture fluid varies little, if at all, is easily prepared, and serves as a favorable substratum for all the cultures used in these tests.

Reaction of the Medium.—The reaction of the test medium in terms of hydrogen ion concentration is of even greater importance. This fact has been entirely overlooked until recently (Wright, 1917). A few preliminary tests showed that not only was the antiseptic power affected, but the specific behavior towards different organisms was also modified. Typical results are given in Table III. The medium given above eliminated this factor, because it had a constant pH of 7.1, the reaction usually favorable for growth of bacteria; also, since it required no adjustment, it was not subject to variation on that account.

TABLE III.

Effect of the Reaction of the Test Medium on the Action of Antiseptics.

Test substance.	Dilution.	Reaction of test medium.*	Test cultures.†				
			2	6	17	12	14
		pH					
Caffeine.	1: 100	6.2	±	±	±	+	±
		7.4	±	±	±	±	−
		8.2	±	∓	±	±	−
Hexamethylenetetramine quaternary salt of *p*-chloroacetylamino-leukomalachite green.	1: 10,000	6.2	−	−	−	+++	±
		7.4	+	+	±	+++	++
		8.2	+	+	+	+++	++
Dibromo-β-naphthol.	1: 12,500	6.2	−	−	−	++	++
		7.4	−	−	−	+++	++
		8.2	−	−	+	+++	+++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	1: 10,000	6.2	−	−	−	−	−
		7.4	−	−	−	+	−
		8.2	−	−	−	++	±

* Nutrient broth was used in all these tests. A quantity of broth was prepared and tubed and N acid or alkali added sterilely to give the desired pH.

† The cultures and symbols are the same as in Table II.

Test Cultures.—The condition of the test culture was the third important factor that had to be considered. Variations are likely to occur either because of lack of adaptation to the test fluid or on account of the inherent fluctuations of the organism itself. To eliminate the former, the cultures were grown in the test broth for at least 3 days, daily subcultures being made; the latter were partly controlled by using wherever possible recently isolated organisms and more than one strain of each type. The strains used, together with their origin and some descriptive remarks, are given in Table IV.

Procedure.—The procedure given below was followed throughout the work. The peptone broth was put up in flasks and autoclaved. 5 cc. portions were then pipetted out sterilely into sterile test-tubes and incubated in a saturated incubator over night. Solutions of the substance to be tested were made up in sterile water and graded amounts added to the broth to give the desired concentration. The stock so-

TABLE IV.

Culture No.	Name.	Source.	Remarks.
123	*B. aerogenes.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; typical.
14	*B.* "	Isolated from stool, 1916.	Indol +; Voges-Proskauer reaction −; not typical; behaves like *B. aerogenes.*
24	*B. cloacæ.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; does not liquefy.
11	*B. coli (communis).*	Isolated from stool, 1916.	Indol +; sucrose −.
13	*B.* " "	" " " 1916.	" +; " −.
12	*B.* " *(communior).*	" " " 1916.	" +; " +.
15	*B.* " "	" " " 1916.	" +; " +.
17	*B. typhosus.*	" " Patient O., 1916.	Agglutinated with typhoid serum. Culturally typical.
19	*B.* "	Isolated from Carrier L., 1916.	
20	*B.* "	Isolated from Carrier C., 1916.	
2	*B. dysenteriæ* Flexner.	Old Institute laboratory stock.	Maltose − (Hiss-Russell).
24	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose + (Flexner).
26	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose − (Hiss-Russell).
6	*B.* " Shiga.	Institute stock strain (Gay).	Reacts typically.
27	*B.* " "	Isolated by Dr. Smillie, summer, 1916.	" "
30	*B.* " "	Albany stock 114 F.	" "
21	*B. proteus.*	Isolated from stool, 1916.	
106	*B. subtilis.*	American Museum of Natural History.	
347	*Staphylococcus aureus.*	American Museum of Natural History.	

lutions were made up of such strength that no more than 1 cc. or less than 0.1 cc. had to be added to give the proper dilution. The cultures to be inoculated were filtered through sterile cotton filters, diluted with broth to give uniform turbidity, and a large standard loop was inoculated into the broth tubes. The tubes were then incu-

bated for 24 hours and the growth was recorded in terms of degree of turbidity. This gave the inhibitive power of the substance in 24 hours. In a few cases the ki ling power in 2 and 24 hours respectively was ascertained by subculturing the broth tubes to agar slants. This procedure was not carried out systematically because of the time consumed and since for the purposes of the study it was sufficient to determine the inhibitive property in a constant time limit.

RESULTS

The results are given in Table V. The compounds used are arranged in the order of their increasing antiseptic power, and the results recorded in terms of the highest inhibiting dilution (first row) and the dilution which just failed to inhibit (second row). These two figures indicate the l'mits of the zone in which the inhibiting dilution lies. Whenever the difference between the two figures was too great, additional tests were made to reduce the gap. When more than one test was made, the average of the results was taken, and when more than one strain of a given organism was used, the average for the type is given. On the whole, individual strains of the typhoid, dysentery, or *aerogenes* bacilli varied but little, while more decided fluctuations were obtained with *Bacillus coli.* The repeated tests checked fairly closely with the original ones.

The facts brought out in Table V are difficult to summarize. In general, it is clear that on starting with aniline or its mono- or dimethyl derivatives, the introduction of a methyl group in the nucleus, as seen in the behavior of the corresponding toluidine derivatives, results in a definite increase in the inhibitive power of the compound. This is also evident from the contrast between quinoline and quinaldine. Similarly, the antiseptic property is enhanced by the substitution of either methyl or ethyl radicals in place of the hydrogens in the NH_2 group. The amount of increase, up to a certain point at least, depends on the number and character of the alkyl radicals introduced. A second alkyl produces a more marked rise than the first, while an ethyl group is more effective than a methyl radical. This general phenomenon is also observed among the dyes. Beginning with fuchsin there is a progressively increasing antiseptic action which

TABLE V.

Inhibition of Growth of Bacteria by Certain Chemical Compounds.

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Methyl alcohol.	10* 20	10 20	5 10	10 20	10 20	10 20			
Ethyl alcohol.	15 25	15 25	10 20	20 25	25 40	25 40			
Aniline.	300 350	250 300	250 300	350 400	450	450	350 400	500 1,000	1,000 5,000
o-Toluidine.	475 550	475 550	475 550	475 550	600 1,000	600 1,000			
p-Toluidine.	475 550	475 550	450 475	550 600	600 650	600 650			
Methyl aniline.	650 700	600 650	600 650	650 700	950 1,000	950 1,000	650 700	1,000 5,000	6,000 10,000
Ethyl aniline.	1,000 1,100	1,000 1,100	1,000 1,100	1,100 1,200	1,350 1,500	1,350 1,500	1,100 1,200		
Methyl *o*-toluidine.	1,100 1,350	950 1,100	950 1,100	1,100 1,350	1,400 1,600	1,400 1,600	1,100 1,350		
Methyl *p*-toluidine.	1,100 1,350	950 1,100	950 1,100	1,100 1,350	1,400 1,600	1,400 1,600	1,100 1,350		

Dimethyl aniline.	1,350 1,500	1,350 1,500	1,350 1,500	1,500 1,750	1,750 2,000	1,750 2,000	1,100 1,350		
Dimethyl *o*-toluidine.	1,550 1,750	1,550 1,750	1,550 1,750	1,750 2,100	2,100 2,500	2,100 2,500	1,350 1,550		
Dimethyl *p*-toluidine.	2,500 2,650	2,650 2,900	2,500 2,650	2,900 3,200	3,500 5,000	3,500 5,000	2,500 2,650		
Diethyl aniline.	4,500 5,400	4,500 5,400	4,500 5,400	6,000 7,000	7,000 8,500	7,000 8,500	4,000 4,500		
Quinoline.	1,400 1,600	1,100 1,400	1,100 1,400	1,600 2,100	1,600 2,100	1,600 2,100	1,100 1,400		
Tetrahydroquinoline.	1,600 2,100	1,400 1,600	1,400 1,600	1,600 2,100	1,600 2,100	2,100 5,000	1,400 1,600		
Quinaldine.	2,600 3,400	2,100 2,600	2,100 2,600	2,600 3,400	2,600 3,400	3,400 5,000	2,100 2,600		
α-Naphthylamine.	2,600 3,100	2,600 3,100	2,100 2,600	2,600 3,100	3,100 7,500	3,100 7,500			
Auramine.	1,800 2,200	2,200 2,700	2,200 2,700	2,700 3,500	2,700 3,500	4,200 5,200	1,500 1,800	6,000 10,000	10,000 25,000
Fuchsin.	8,000	8,000 12,000	12,000 15,000	12,000 15,000	100,000 110,000	110,000 200,000	12,000 150,000	500,000 1,000,000	300,000 500,000
Malachite green.	20,000	40,000 45,000	45,000 50,000	30,000 35,000	250,000 500,000	500,000 1,000,000	35,000 40,000	4,000,000 5,000,000	1,000,000 2,000,000

* The numbers indicate dilutions; the first row the inhibiting dilution, the second the one which failed to inhibit.

TABLE V.—*Concluded.*

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A.	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Victoria green.	40,000	60,000 70,000	70,000 80,000	40,000 50,000	700,000 1,400,000	700,000 1,400,000	40,000 50,000	5,000,000 10,000,000	1,000,000 2,000,000
Dahlia.	45,000	55,000 65,000	65,000 75,000	45,000	110,000 150,000	110,000 150,000	55,000 65,000	2,000,000 4,000,000	800,000 1,000,000
Methylviolett B.	60,000	70,000 75,000	75,000 85,000	60,000 65,000	175,000 250,000	175,000 250,000	85,000 95,000	4,000,000 5,000,000	800,000 1,000,000
Gentian violet.	25,000 50,000	85,000 95,000	90,000 100,000	50,000 60,000	100,000 200,000	100,000 200,000			
Crystal violet.	60,000	85,000 95,000	100,000 110,000	85,000 95,000	400,000 800,000	400,000 800,000	200,000 250,000	4,000,000 5,000,000	1,000,000 2,000,000
Brilliant green.	100,000 175,000	675,000 800,000	550,000 600,000	510,000 550,000	1,500,000 2,000,000	2,000,000 3,000,000	675,000 800,000	15,000,000 20,000,000	4,000,000 5,000,000
Methyl green.	1,200 2,200	1,200 2,200	1,200 2,200	1,200 2,200	8,000 15,000	8,000 15,000	2,700 3,500	50,000 100,000	30,000 50,000
Aniline blue.	1,200	1,200	1,200	1,200	1,200	1,200	1,200		

runs parallel with the increase in the number of methyl or ethyl groups. The triethyl derivative is about as effective as the hexamethyl, while the tetraethyl is the most active of the series. The behavior of the *o*- and *p*-dimethyl toluidines indicates that position may be a factor in determining the degree of antiseptic action. The effect of the introduction of chlorine into the benzene nucleus is seen from the differences between malachite green and victoria green. This effect on the introduction of halogen has been observed before in other classes of compounds. It is also interesting to note that the substances containing two aromatic nuclei, namely naphthylamine, quinoline, quinaldine, and the diphenylmethane dye, auramine, are more potent than the corresponding monophenyl derivatives, whereas the triphenyl derivatives are the most active of the substances tested.

It would seem, then, that the inhibiting effect of these substances is due on the one hand to aniline with the benzene nucleus as its basis, and on the other to the number of these nuclei. The effect is consistently enhanced by the addition of alkyl radicals, either to the nucleus, or to the amino group. The number and character of these radicals also determine the degree of effectiveness.

An exception to the general phenomenon of the increase of inhibitive action produced by the increase in the number of alkyl groups is seen in the anomalous behavior of methyl green. This substance is identical with crystal violet, with the exception that one of the tertiary nitrogens of this dye has been changed as a quaternary salt by the addition of methyl chloride. Contrary to the expectation of an increase in antiseptic power, this dye is almost inert. It is also noteworthy that in the case of the triphenyl derivative of rosaniline, aniline blue, in which the hydrogens are substituted by phenyl groups, there is a decided reduction in inhibitive action.

In some respects, these results are in accord with those obtained by other workers with other classes of compounds. The well known difference between phenol and cresol and the observations of Jacobs, Heidelberger, and Bull of the progressively increasing bactericidal action, on proceeding from the dimethyl to the diethyl and dipropyl derivatives of certain quaternary salts of hexamethylenetetramine, may be cited as instances.

While it is possible to point to the factors concerned in the enhancement of the germicidal power, it is difficult to account for the specific behavior of these substances. All the compounds tested are decidedly more active against the Gram-positive than the Gram-negative bacteria. This is true as well of aniline and its methyl derivative as of auramine and the triphenylmethane dyes. It is interesting to note that while aniline and its derivatives are more active against *Staphylococcus aureus* than *Bacillus subtilis*, the converse is true of the dyes.

Partial specificity becomes most marked in the triphenylmethane dyes. This is particularly evident in their more potent action against the Gram-positive organisms and the dysentery bacilli. Though the dysentery bacilli are exceedingly sensitive to the action of these substances, they are decidedly less so than the Gram-positive bacteria. These dyes are also markedly inactive against *Bacillus aerogenes* and, with the exception of fuchsin, are more inhibitive for *Bacillus coli* than for *Bacillus typhosus*.

The fact that all these dyes behave alike, irrespective of the number and character of the alkyl radicals, indicates that the molecule as a whole is concerned with the partial specific action. This is in accord with the fact that three of these dyes have been used in the isolation of *Bacillus typhosus*.

Mention should be made of the side-light which the behavior of these organisms towards this group of substances throws on their possible relationship. *Bacillus typhosus* and *Bacillus aerogenes* (also *Bacillus paratyphosus* B) are more sensitive to the simple aniline derivatives and less so to the dyes than *Bacillus coli*. The latter, as well as *Bacillus dysenteriæ*, is relatively much more sensitive to the dyes than to the aniline compounds. The extreme sensitiveness of the dysentery bacilli to the action of the dyes is especially interesting. While there was little difference between the behavior of the two classes, the Flexner cultures invariably showed a greater tolerance for fuchsin than did the Shiga bacillus. Their extreme sensitiveness to this class of chemical compounds renders it unlikely that any representative of the group may be found that will be of service in isolating them from polluted materials.

CONCLUSIONS.

This study of the inhibitive effect of aniline and some of its derivatives and of the triphenylmethane dyes on certain bacteria warrants the following tentative conclusions:

1. The composition and reaction of the medium exert a marked influence on the behavior of the antiseptic. The higher the concentration of organic nitrogenous compounds (peptone) in the medium, the lower is the effective concentration of the dye. The reaction of the medium modifies the specific action of the antiseptic, owing probably to an alteration in the bacterial cell.

2. The germicidal action of the compounds is a function of the benzene nucleus, the added elements or radicals, their number, and, in the case of the dyes, probably the quinoid structure of the nucleus.

3. As far as tested, the increase in the number of alkyl radicals increases the antiseptic power. Methyl green is an interesting exception to this rule, for the change of one of the nitrogens to the quaternary salt is accompanied by an almost complete loss in inhibitive action.

4. The antiseptic power is enhanced to a greater extent by an ethyl than a methyl group, and the second alkyl produces a proportionately greater increase than the first. It appears that the relative position of the introduced group may be a factor in determining the relative improvement in the effectiveness of the compound.

5. The introduction of a methyl group in the nucleus consistently enhances the inhibitive action of the compound and its alkyl derivatives. This is evident from a comparison of the action of aniline and its derivatives with that of toluidine and its corresponding derivatives.

6. The simple aniline derivatives, as well as the dyes, are more toxic for the Gram-positive than the Gram-negative bacteria. Of the former, *Bacillus subtilis* is more sensitive to the dyes than *Staphylococcus aureus*, while the reverse is true in the case of the aniline compounds.

7. The most marked specific selective effect is manifested by the triphenylmethane dyes. *Bacillus aerogenes* and *Bacillus typhosus* possess a higher resistance to these substances than *Bacillus coli* or

Bacillus dysenteriæ. The last is exceedingly sensitive. This partial specificity is apparently a function of the molecule as a whole.

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THE ELIMINATION OF IRON AND ITS DISTRIBUTION IN THE LIVER AND SPLEEN IN EXPERIMENTAL ANEMIA. II.

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In an earlier communication (1) we presented the results of a study of the elimination and storage of iron in transient experimental anemias due to a single injury. These we found were not characterized by an increased elimination of iron in the feces, or in the urine, except as an accompaniment of hemoglobinuria. On the other hand, an increased storage of iron in the liver and spleen was constantly observed. These observations support well known views concerning the power of the body to conserve iron, and suggest that some other factor besides blood destruction must be operative in those hemolytic anemias in man characterized by excessive elimination of iron in the feces. The natural assumption is that in the hemolytic anemias there exists some disturbance of the mechanism concerned in the retention or conservation of iron, and we have now studied a chronic type of experimental anemia in order to determine whether chronicity may be a factor. In the present series of experiments *Trypanosoma equiperdum* has been used to cause a continuous blood destruction in dogs. It was hoped that in the long continued and progressive anemia so produced we might find an experimental condition approximating the hemolytic anemias of man.[1]

EXPERIMENTAL.

As our methods of experimentation and analysis have been described in a previous paper (1), they need not again be given. The

[1] Only the study of iron metabolism is presented here. For a general description of these experiments see Krumbhaar, E. B., Experimental trypanosomiasis: *T. equiperdum* infection in the dog, *J. Infect. Dis.*, in press.

TABLE I.

Iron Elimination in Urine and Feces in Trypanosome Anemia with and without Splenectomy.

Dog No.	Date of infection.	Blood picture.			Period.	Iron.			Remarks.
		Date.	Hemoglobin.	Red blood corpuscles.		Urine per day.	Feces per day.		
			per cent			*mg.*	*mg.*	*per cent*	
1	Nov. 1 and 17	Nov. 2	94	6,550,000	Nov. 1–7	1.39	20.7	0.138	No splenectomy.
		" 7	72	5,220,000					
		" 28	42	3,550,000	Nov. 28–Dec. 4	1.48	18.6	0.118	
		Dec. 4	48	4,980,000					
2	Dec. 15	Nov. 11	99	6,880,000	Nov. 14–20	1.68	23.7	0.123	No splenectomy. Dec. 19. Fragment of spleen removed.
		Jan. 8	61	3,660,000	Jan. 9–15	1.59	24.7	0.128	
3	Jan. 11	Jan. 13	115	7,800,000					No splenectomy.
		" 27	84	4,720,000	Jan. 23–27		18.6	0.103	
4	Jan. 8	Jan. 13	111	7,400,000					No splenectomy.
		" 27	93	5,900,000	Jan. 23–27		15.8	0.109	
5	Nov. 1	Nov. 2	99	7,280,000	Nov. 1–7	1.43	18.7	0.131	
		" 6	82	6,240,000					
		" 28	45	3,600,000	Nov. 28–Dec. 4	1.63	18.0	0.105	
		Dec. 4	40	3,680,000					Dec. 13. Splenectomy.
		" 18	44	3,510,000	Dec. 18–22	6.08*	12.8*	0.099	
6	Jan. 23	Dec. 9	94	6,520,000	Dec. 9–15		24.7	0.114	Feb. 10. Splenectomy.
		Feb. 13	30	2,900,000	Feb. 12–16		20.4	0.119	
7	Control.	Nov. 11	105	7,100,000	Nov. 14–20	1.20	15.4	0.127	Nov. 27. Splenectomy.
		Dec. 4	104	7,480,000	" 28–Dec. 4	1.39	16.8	0.112	
8	Control.	Jan. 10	107	7,440,000	Jan. 9–15		29.6	0.100	Jan. 22. Splenectomy.
		Feb. 14	104	7,050,000	Feb. 10–16		30.1	0.111	
9	Control.	Jan. 10	92	6,900,000	Jan. 9–15		19.7	0.120	Jan. 22. Splenectomy.
		Feb. 14	88	6,410,000	Feb. 10–16		20.1	0.117	

* Feces mixed with urine.

results of the study of the elimination of iron in the feces are presented in Table I, in which are given also some figures for the urine. The urine was not studied in all experiments inasmuch as our earlier work demonstrated that in the absence of hemoglobinuria the iron content of urine does not vary. Six of the animals were infected with trypanosomes and two of these were splenectomized.[2] In addition the table presents three control experiments on non-infected animals with studies of iron elimination before and after splenectomy.[3] The figures are in accord with those obtained in our earlier study (1) of transient anemia and prove conclusively that an experimental anemia with continuous blood destruction is not necessarily characterized by an increased elimination of iron in the feces. In this connection we consider Experiments 5 and 6, in which splenectomy was done at the stage of severest anemia, very important. The removal of the spleen of the normal dog, does not, as has been shown in other work from this laboratory (2), lead to an increased elimination of iron, but no observations are at hand concerning iron elimination after removal of the spleen in an anemic dog. Experiments 5 and 6 offer the needed evidence to show that iron elimination after splenectomy, in the anemic as in the normal dog, remains unchanged.

In Table II may be seen the figures showing the distribution of iron in the spleen and liver. The normal percentages for spleen and liver, as we have shown elsewhere (1), are respectively 0.185 and 0.104. The figures for trypanosome anemia are sometimes higher, but in general are in accord with those obtained in the study (1) of transient anemia. Experiments 1, 2, 3, 4, 10, and 11 are simple studies of trypanosome anemia. In Experiments 12, 13, and 14 arsenobenzol,[4] a trypanocide, was given to delay the fatal termination in the hope of obtaining a greater accumulation of iron in the liver and spleen. These animals thus suffered a severe initial anemia, an improvement as the trypanosomes disappeared under treatment, and eventually,

[2] These operations were done under complete ether anesthesia.

[3] The averages of all figures for iron elimination in the earlier study are as follows: urine, 1.6 mg. per day; feces, 18.9 mg. per day, or 0.116 per cent of dry weight.

[4] The arsenobenzol (Schamberg) was furnished by the Dermatological Research Laboratories of the Polyclinic Hospital, Philadelphia.

TABLE II.

Iron in Liver and Spleen in Experimental Trypanosome Anemia.

Dog No.	Date of infection.	Degree of anemia *			Iron in the dry organ.		Remarks.
		Date.	Hemoglobin.	Red blood corpuscles.	Spleen.	Liver.	
			per cent		per cent	per cent	
1	Nov. 1 and 17	Dec. 7	48	4,980,000	0.270		Dec. 19. Fragment of spleen removed.
2	Dec. 15	Jan. 16	56	3,900,000	0.285	0.101	Jan. 18. Autopsy.
3	Jan. 11	Feb. 6	72	3,700,000	0.573	0.186	Feb. 9. "
4	" 8	" 6	50	4,570,000	0.478	0.176	" 6. "
10	Feb. 6	" 28	68	4,700,000	0.632	0.217	Mar. 2. "
11	Mar. 22	Mar. 31	90	3,130,000	0.466	0.133	Apr. 5. "
12	Feb. 6†	May 1	40	4,000,000	0.293	0.224	May 6. " Feb. 29, Mar. 1 and 2. Salvarsan.
13	Apr. 22†	June 5	55	3,690,000	0.431	0.235	June 6. " May 16–24. Salvarsan.
14	" 22†	May 29	54	3,540,000	0.315	0.186	" 6. " " 16–22. "
5	Nov. 1	Dec. 18	44	3,510,000	0.274‡	0.104	Dec. 13. Splenectomy. Dec. 22. Autopsy.
6	Jan. 23	Feb. 13	30	2,900,000	0.422‡		Feb. 10. " Feb. 17. "
7	Dec. 15	Nov. 27	112	7,240,000	0.179§	0.178	Nov. 28. " Dec. 22. Died. Autopsy.
8	Mar. 28	Jan. 24	110	7,700,000	0.180§		Jan. 22. " May 11. Autopsy.
		May 1	35	3,940,000		0.290	
9	Apr. 2	Jan. 20	96	7,200,000	0.174§	0.117	" 22. " Apr. 5. "
15	Mar. 9	Mar. 26	45	3,920,000	0.455‡	0.159	Mar. 26. " Mar. 29. "
16	Apr. 22	May 26	23	2,610,000	0.605‡	0.171	May 22. " May 27. Died.
17	Feb. 2†	Mar. 3	72	5,090,000	0.353‡		Mar. 2. " Apr. 7. Autopsy. Mar. 9. Salvar-
		Apr. 3	20	2,590,000		0.264	san.
18	Apr. 21	May 5	60	5,150,000	0.181§	0.181	Apr. 13. Splenectomy. May 4. Autopsy.
19	" 21	" 1	90	7,880,000	0.190§	0.135	" 13. " " 7. "

* The degree of anemia represents blood examination most nearly approximating in point of time the removal of the organ.

† In this animal treatment by salvarsan caused a disappearance of trypanosomes and improvement of the anemia but with eventual return of parasites in the circulating blood and recurrence of anemia.

‡ Splenectomy followed infection.

§ Splenectomy preceded infection.

with the reappearance of the parasites in the circulating blood, a recurrence of anemia, terminating fatally. Despite the long course of the experiment the storage of iron was not greatly augmented in either liver or spleen. The results, in general, are in accord with the work of Boycott and Price-Jones (3), who, in a study of experimental trypanosome anemia in the rabbit, found the iron of the liver to be double that of normal animals while the iron of the spleen was increased twenty times.

In Experiments 7, 8, 9, 18, and 19 the spleen[2] was removed before, and in Experiments 5, 15, 16, and 17 after infection to see whether the absence of the spleen led to greater storage of iron in the liver. No evidence of this is at hand.

TABLE III.

Iron Elimination in Bile.

Dog No.	Period.	Iron.			Remarks.
		Urine-bile mixture per day.	Feces per day.		
		mg.	*mg.*	*per cent*	
20	Nov. 1–7	1.84	30.4	0.109	Nov. 1. Bile duct-ureter anastomosis.
	" 11–17	1.97	36.4	0.123	
21	Nov. 1–7	1.75	7.3	0.119	Nov. 1. Bile duct-ureter anastomosis.
	" 11–17	1.91	6.7	0.118	
	" 28–Dec. 4	1.51	10.0	0.127	Nov. 24. Splenectomy.

In seven animals with anemia the iron content of the kidney was determined, the figures varying from 0.044 to 0.067 per cent of dry substance, essentially normal figures.

In another series of experiments, the elimination of iron in the bile was studied, in order to determine whether a loss occurred through this channel. For the collection of bile we used the method of Pearce and Eisenbrey (4), which consists in an anastomosis, after removal of one kidney, of the corresponding ureter with the bile duct.[2] By this procedure the bile passes into the urinary bladder and is available, without loss, for the quantitative chemical examination of any constituent not present in the urine. As we had at hand many control

figures for iron in the urine, there was no possibility of confusion as to figures for iron in the urine and bile respectively. In Table III are shown the results in two experiments. The figures for urine and bile combined approach so closely those for urine alone that this line of study was abandoned with the conclusion that in the dog iron in appreciable amounts is not eliminated in the bile.

However, in the animals with bile duct-ureter anastomosis an increased storage of iron was found in the spleen. As anemia was not

TABLE IV.

Iron Storage in the Spleen after Exclusion of Bile from the Intestine.

Dog No.	Blood picture.			Iron in spleen.	Remarks.
	Date.	Red blood corpuscles.	Hemo-globin.		
			per cent	*per cent*	
20	Nov. 7	6,820,000	103		Nov. 1. Bile duct-ureter anastomosis.
	Dec. 14	7,600,000	105	0.498	Dec. 19. Splenectomy.
21	Nov. 7	7,100,000	102		Nov. 1. Bile duct-ureter anastomosis.
	" 17	6,680,000	108	0.548	" 24. Splenectomy.
22	Feb. 7	6,020,000	86		Jan. 23. Bile duct-ureter anastomosis.
	Apr. 14	5,820,000	92	0.253	Apr. 17. Splenectomy.
23	Mar. 27	6,860,000	92		Feb. 6. Bile duct-ureter anastomosis.
	May 22	3,830,000	35	0.472	Anemia due to toluylenediamine.
					May 24. Autopsy.
24	Apr. 14	6,610,000	110		Jan. 24. Bile duct-ureter anastomosis.
	May 15	4,690,000	45		Anemia due to toluylenediamine.
	" 19	5,210,000	75	0.598	May 24. Autopsy.

produced artificially and did not develop spontaneously in three animals of the series this increased storage of iron is difficult to explain. It is possible that the loss of bile from the intestine may have been a factor, but as a satisfactory explanation is not at hand, the results are given in Table IV without further comment.

SUMMARY.

In the continuous blood destruction, essentially a chronic experimental anemia, caused by infecting the dog with *Trypanosoma equi-*

perdum, no increased elimination of iron is observed in the feces. The storage of iron in the liver and spleen under these experimental conditions is somewhat greater in amount, but of the same general character as in transient experimental anemia.

Splenectomy before or after infection, *i.e.* the development of anemia, influences neither the elimination of iron in the feces nor its storage in the liver.

The retardation of the course of the trypanosome infection and thus the production of a more chronic anemia by treatment with a trypanocide, arsenobenzol, likewise does not affect iron storage.

These experiments have therefore failed to reproduce the changes in iron metabolism seen in certain of the chronic hemolytic anemias of man.

In the presence of a bile duct-ureter fistula the iron content of the mixture of urine and bile is not appreciably greater than that of the urine alone. In the dog, therefore, the elimination of iron in the the bile would not appear to be an important factor.

On the other hand, when bile is excluded from the intestine an unusual storage of iron occurs in the spleen. For this no explanation is offered.

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A STUDY OF THE BLOOD PRESSURE BY THE METHOD OF GAERTNER, ESPECIALLY IN PATIENTS SUFFERING FROM FIBRILLATION OF THE AURICLES.

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INTRODUCTION.

A number of years ago Mackenzie (1) called attention to the difficulty in estimating the blood pressure in persons suffering from fibrillation of the auricles. There was general agreement that the difficulty existed, but there seemed to be no method for improving the technique of making clinical determinations. The beats which were heard first by auscultation or felt first on palpation, when pressure in the cuff system was allowed to escape, continued to be taken as the measure of the systolic pressure. A method was then suggested by James and Hart (2), later called the fractional method by Kilgore (3), by which not the maximum pressure of a few beats is taken as the measure of pressure, but the average of the systolic pressures of all the beats. Their technique consists in counting for 1 minute the number of beats felt below the brachial cuff at the radial artery at levels 10 mm. of mercury apart. The figure obtained is multiplied by the level of pressure, all the products are then added, and the sum is divided by the apex rate. Slight modifications and certain extensions of the method have since been suggested by Kilgore. He employed auscultation in addition to palpation, especially in calculating diastolic pressure.

This method presents certain difficulties, but before considering them we wish to review again certain points underlying the current clinical technique of estimating blood pressure. The peripheral arteries are regarded as representing an elastic reservoir kept filled by the action of the heart. It is the tendency of this reservoir to return to a position of rest, and in doing so to empty itself continuously into the smallest arteries and capillaries. By this effort these vessels are kept constantly filled.[1] So far as the constancy of the filling is concerned it makes no difference whether the elastic reservoir is filled by

[1] Cases in which there is a capillary pulse are exceptions to this rule.

volumes of blood equal in amount as normally, or unequal as in fibrillation of the auricles; or whether these volumes are delivered at equal or at somewhat unequal intervals. It matters only that the total volume in succeeding units of time, minutes for instance, is kept constant. Under ordinary circumstances this maintenance of constant volume is accomplished by uniform filling, but, failing this, it can be brought about by a compensating increase (or decrease) of resistance offered at the outflow of the reservoir system. It is an advantage to have the minute volume output of the left ventricle divided in roughly equal fractions throughout the minute. The point at which the reservoir empties into the smaller vessels may be regarded as the point where the head of pressure in the reservoir becomes effective. We speak then of the "effective pressure"[2] at this point.

The clinical habit of estimating arterial pressure in the brachial artery is adopted, not because the pressure here is especially important, but because it serves, on the one hand, as a rough indicator for the pressure level at the root of the aorta or in the left ventricle itself; that is, a difference is assumed between aortic and brachial pressure, sufficiently constant to permit the brachial pressure to serve as a valuable guide. On the other hand, brachial pressure may also serve as a suitable guide for judging the level of effective pressure, the pressure at the exit from the arterial into the capillary system. So far as the capillary system is concerned, there is no difficulty in this convention as long as the filling of the reservoir is maintained by even and uniform strokes; when the strokes are no longer uniform as in fibrillation, the difference between effective pressure, which tends to stay constant or alters only within narrow limits, and brachial pressure, which varies, must fluctuate so that the brachial pressure can no longer serve as an index of effective pressure.[3]

These considerations are important in relation to the technique of estimating systolic pressure when the auricles are fibrillating, for it is precisely in this condition that brachial pressure fluctuates and fails as an index of effective pressure. It has been found on closer study (3) that in auricular fibrillation not only the systolic, but also

[2] This phrase is used by James and Hart (2).

[3] See experiments reported by Cohn, A. E., and Lundsgaard, C., *J. Exp. Med.*, 1918, xxvii, 505.

the diastolic pressure of succeeding beats varies. These fluctuations necessarily result in irregularities in the pulse pressure. The pulse pressure does, in fact, undergo large alterations in the brachial artery. Further toward the periphery, however, the pulse pressure becomes progressively smaller, until, in the capillaries, it ceases to exist[1] and the flow is constant and continuous. The point at which this takes place is the point where, as has been said, the head of pressure becomes effective. Here a constant reading can be obtained. But since for clinical purposes no practicable technique exists for this, a point just proximal to the capillaries may be chosen. In the small arteries, of the size of the digital, the pulse pressure is small and alterations from mean pressure can from beat to beat be shown to be relatively unimportant.[4] For taking the pressure at this point a technique already exists. It is the method formerly employed by Gaertner and described by him in 1899 (4). The values obtained may be regarded as effective blood pressure. Readings so obtained are direct and the technique is simple. By this method we have taken the blood pressure of a few persons suffering from fibrillation of the auricles and have plotted, parallel to these curves, others made day after day by the fractional method of James and Hart, with the view of ascertaining the difference between the brachial and digital pressures. We have, in addition, studied a number of other individuals, some with normal and some with abnormal hearts, but all having hearts the mechanism of which was normal.

Technique.

The following technique was employed. One finger,—the ring finger,—was rendered bloodless by rolling a thick rubber ring from the tip to a small pneumatic cuff which was applied to the first (base) phalanx. The cuff corresponded exactly in plan to that of von Recklinghausen except that it was 2.5 cm. wide and about 5 cm. long. The cuff was connected by pressure tubing to a mercury manometer as in the von Recklinghausen plan. The pressure in this system was raised with a pump after the finger was blanched by the rubber ring. The ring was then removed and the pressure in the manometer cuff system

[4] This point is described in our report, *J. Exp. Med.*, 1918, xxvii, 505.

allowed to fall gradually and regularly.[5] When the pressure in this system falls to the level of the blood pressure, blood begins to flow into the finger distal to the cuff and is recognized by the return of color in the finger. After a little practice there is no difficulty in recognizing the return of color. It is important to make the estimations with north light. We have been aided in seeing the return of color, even in the very anemic, by laying the finger and hand to be examined on a dark, blue-gray cloth, to provide a proper contrast. It was the custom to read the pressure in each case 10 times and to average the readings. Of 153 determinations the range was 1 to 5 mm. in 11 determinations; 6 to 8 mm. in 69; 9 to 10 mm. in 35; 11 to 15 mm. in 27; 16 to 30 mm. in 7; 21 to 25 mm. in 2; and 26 to 30 mm. in 2. That is, the range was below 10 mm. in 75 per cent of the determinations. In many instances both observers made independent readings. When that was done, we compared the averages in twenty-six instances and found a difference of 2 to 4 mm. Twice the averages differed by 5 mm.; twice by 6 mm.; once by 7 mm.; twice by 8 mm.; once by 9 mm.; and once by 11 mm. The differences in range are greater than were found in the estimation of brachial pressure by Kilgore and his associates (5) but not so great as to render the method unserviceable. As an example of the method followed, we cite the figures of one patient, Case 2334 (Tables I and II), made by two observers.

Studies of blood pressure by Gaertner's method have been made with the view of comparing the readings found at the digital, with those taken at the brachial artery.

Hayashi (6), in Strümpell's clinic, found a difference of about 20 mm. between the readings taken of these two arteries whether they were made in children, men, or women, in infections, or in cardiac, vascular, or renal diseases. There were fluctuations in his figures but these were not of sufficient importance to alter the conclusion that the fall in pressure from the brachial to the digital vessels was about 20 mm. Doleschal (7) compared the pressure in the radial artery

[5] We provided for the gradual and regular fall in pressure by placing the rubber bulb which served as a pump between the jaws of a wooden vice. The distance between the jaws was regulated by a screw turned by a long shank; the use of a long shank permits more uniform motion. As the screw was released, the jaws of the vice were separated by springs properly placed.

TABLE I.

March 26, 1917.

Tonometer readings.	
No. 1.	No. 2.
mm. Hg	*mm. Hg*
93	93
89	95
92	95
88	95
87	93
91	93
94	92
95	93
94	95
93	92
Average...........91.6 = 92^{+3}_{-4}	93.6 = 94^{+1}_{-2}

TABLE II.

Blood pressure by the fractional method.			
Auscultation.		Palpation.	
Pressure.	No. of beats heard	No. of beats felt.	Pressure.
mm. Hg			*mm. Hg*
140	0	0	
130	31	20	130 × 20 = 2,600
120	25	22	120 × 2 = 240
110	9	26	110 × 4 = 440
100	9	61	100 × 35 = 3,500
			6,780
90	21	59	
80	18	61	
70	7	58	
60	2	60	$\frac{6780}{62} = 109$
50	0.	56	
Average pressure...............130		109	
Radial rate.................. 62			
Apical " 62			

taken by means of von Basch's sphygmomanometer with that taken by Gaertner's tonometer and found uniformly that the tonometer readings were lower than those taken with von Basch's instrument. These results were naturally to be expected, and show that even where the pressure differences are probably small, as in the case of the radial and digital arteries, the tonometer readings, as we anticipate, are lower. Of 200 cases, for instance, the readings showed differences up to 5 mm. in 89, to 10 mm. in 80, to 15 mm. in 19, to 20 mm. in 6, and from 20 to 40 mm. in 6.

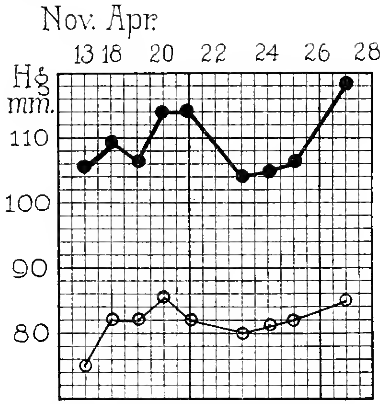
Our experience corresponds closely with that of Hayashi and Doleschal, for we have found uniformly that the tonometer readings at the digital arteries are always lower than the brachial readings. In eight normal individuals we found the average difference to be 20 (19.8) mm., the maximum being 30 mm., the minimum 5 mm. (Table III).

TABLE III.

No. of individual.	Brachial systolic pressure by auscultation.	Tonometer.	Difference.
	mm. Hg	*mm. Hg*	*mm. Hg*
1	110	96	14
2	120	92	28
3	116	97	19
4	119	98	21
5	110	96	14
6	114	109	5
7	120	90	30
8	109	81	28
Average.			19.8

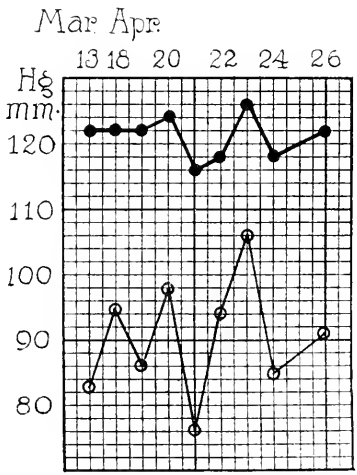
In the patients suffering from chronic heart disease and chronic nephritis (Table IV), in whom the mechanism of the heart beat was normal, the average difference between the two was 25 mm. in all cases, the range being from 6 to 80. If two cases (Nos. 10 and 12), suffering from complete heart block, are omitted, the average is 18 and the range 6 to 30. These values do not differ from normal.

If the cases are arranged according to the height of the brachial pressure, no correspondence between this and digital pressure is observed. The reason probably depends on the instability known to exist in the vasomotor mechanism in different individuals. It is pre-



——— Brachial pressure estimated by the method of auscultation.
– – – Brachial pressure estimated by palpation.
——— Digital pressure.

TEXT-FIG. 1. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.



TEXT-FIG. 2. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.

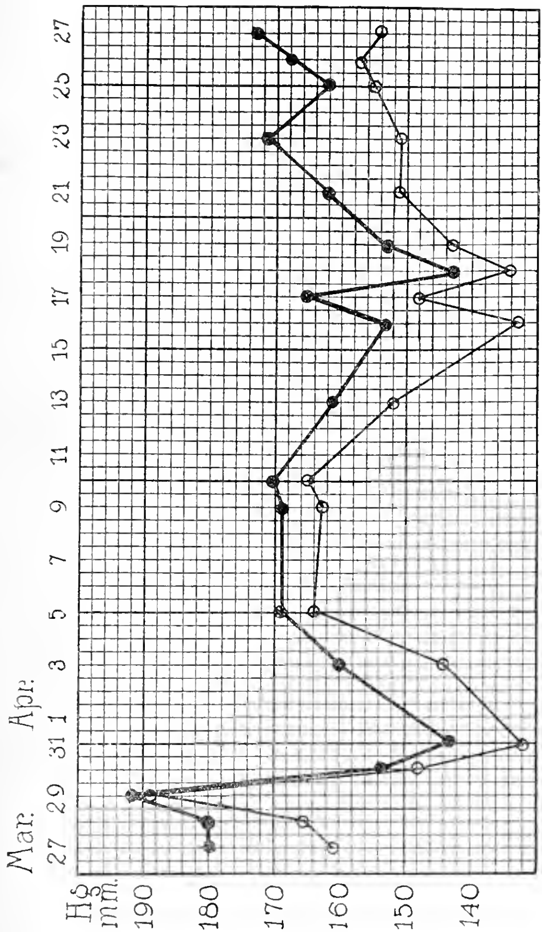
cisely in the smaller vessels that lability is observed, so that fluctuation is expected in the digital rather than in the brachial artery. It is for this reason that the readings in the two arteries are not parallel. In some respects the violent fluctuations in digital pressure present difficulties, so that as a guide for comparison pressure in the digital artery is unsatisfactory. The question arises, however, whether the difficulty which is found is not of clinical importance, and whether it ought not to be emphasized rather than evaded entirely as is now done in estimating pressure only at a point such as the brachial ar-

TABLE IV.

Case No.	Hospital No.	Rate.	Brachial systolic pressure.	Tonometer.	Difference.
			mm. Hg	*mm. Hg*	*mm. Hg*
1	587	80	84	65	19
2	2915	86	100	84	16
3	2069	109	103	73	30
4	2681	96	145	126	19
5	2867	83	134	106	28
6	2862	82	145	128	17
7	2961	90	153	147	6
8	2907	82	160	139	21
9	2336	85	176	169	7
10	1266	30	176	135	41
11	2992	92	180	162	18
12	2833	30	250	170	80
Average........					25.1

tery where the least variation is found. We recall that the method of Gaertner was practically abandoned because of the fluctuations inherent not so much in the technique as in the artery itself. Furthermore, the matter is important on account of questions associated with fluctuations in oxygen unsaturation in the venous blood of the arm such as Lundsgaard (8) has found. These phenomena may find their explanation, in part, in facts like those which are shown here.

Curves of the brachial and digital pressure of two individuals in both of whom the circulation was normal illustrate these points. In the first instance (Text-fig. 1) the pressure in the brachial artery fluctuated within the usual narrow limits from 104 to 118 mm., a range of



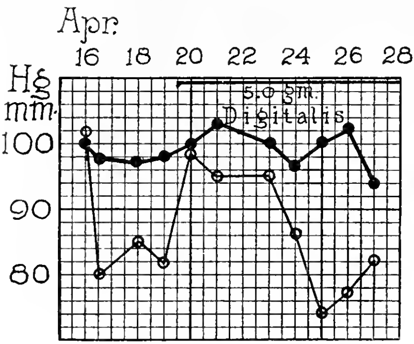
TEXT FIG. 3. Curves of the brachial and digital pressure of a patient suffering from chronic nephritis and heart failure.

14 mm. Parallel digital pressure fluctuated between 75 and 85 mm., a range of 10 mm. The digital pressure was more constant than the brachial. In the second instance (Text-fig. 2) the brachial pressure fluctuated between 116 and 126 mm., a range of 10 mm., while the digital pressure fluctuated between 76 and 106 mm., a range of 30 mm., or three times as great as the brachial pressure. We expected the peripheral circulation of the first individual to be stable, but in the second we anticipated, because of the frequent rapid alterations of vasomotor tone seen in phenomena like dermatographia, that it was labile. Studies of blood flow, or oxygen unsaturation, already mentioned, had indicated that phenomena of the nature we are now emphasizing, existed to explain the obscure facts relating to flow. There is reason, therefore, to think that in studies like this, facts of importance may be found.

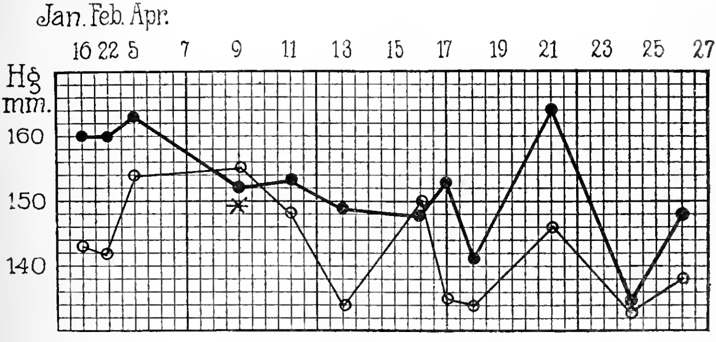
In this connection a consideration of the records of a patient (Text-fig. 3) are important. This individual suffered from chronic nephritis and heart failure; he was at first edematous and orthopneic and had a high pressure and an alternating pulse. The digital pressure fluctuated between 132 and 189 mm., a range of 57 mm. But the brachial systolic pressure fluctuated almost as violently between 143 and 192 mm., a range of 49 mm. In this instance the fluctuations are almost parallel and indicate that they depend on alterations in the functional state of the heart rather than on fluctuations in vasomotor tone. These three cases show that there are instances in which simultaneous brachial and digital readings are of value. But we have cited them in addition to show that of the two arteries, the blood pressure is uniformly higher in the one more centrally placed; the curves of the two do not cross.

OBSERVATIONS.

Our especial interest was directed to the study of the relation of digital to brachial pressures in individuals suffering from fibrillation of the auricles. We studied four patients in detail. The pressure curves made by the tonometer method are not remarkably different from those found when the mechanism of the heart beat is normal. But when the digital pressure curves are compared with the brachial pressures obtained by the method of fractional readings devised by



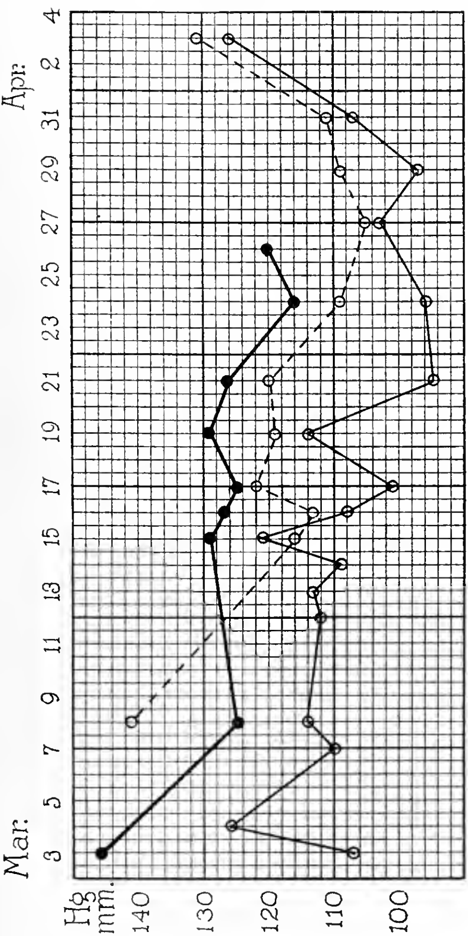
Text-Fig. 4. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once.



Text-Fig. 5. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross twice. The upper curve represents pressure in the brachial artery determined by the method of auscultation, except where indicated by an asterisk. The lower curve represents digital pressure taken by Gaertner's method.

James and Hart, difficulties occur. In the first place, the taking of readings requires as many minutes or half minutes as there are levels at which pressure is read. During this time the state of the vessels does not remain the same, the number of sounds heard or beats felt at one level may, after 3, 4, or 5 minutes, alter appreciably; sometimes, indeed, no reliable count is possible. To avoid continuous pressure, we invariably reduced the pressure to zero, for at least 1 minute between successive readings, although the length of the examination was thereby doubled. The procedure, however, did not remove the difficulty. The explanation for this may depend on a number of causes related to the auscultatory method, possibly to the formation of the sounds. But another cause also deserves considertion. Compression of the brachial vessels for so long a time may alter the partition of the blood stream so that an increasing fraction passes, uncompressed by the cuff, in the medullary vessels of the bone or in vessels protected by the grooves on its surface. This explanation implies a rapid increase in the area of the collateral vessels, but there is reason for believing that it may take place. It has, on the other hand, been observed that the difficulty may develop gradually. If absent at first, it increases as the daily examinations proceed. The establishment of the collateral circulation need, therefore, not be abrupt. The factors involved in the production of the sounds, whether produced by the mechanism of the water-hammer or otherwise, may also change. Whether these are the occurrences which actually take place and are the ones responsible for the difficulty we are considering, is of secondary importance. The sounds in any event do not remain sufficiently distinct in many individuals to make auscultation reliable as the basis of the fractional method. For the reasons stated, counting the beats by palpating the radial artery also offers difficulties. We have, therefore, in two cases counted the rate in the radial artery by palpation as well as in the brachial by auscultation. In one case in which it was satisfactory, we used the auscultatory method alone.

A second difficulty is that the curve of average brachial pressure occasionally crosses and is lower than the level of digital pressure taken on the same day. A crossing of the two curves had not been observed by others and was not seen in our studies of individuals the



TEXT-FIG. 6. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once when the fractional pressure by palpation is considered.

mechanism of whose hearts was normal. The crossing of the curves is, moreover, inconceivable. In Case 13 (Table V) crossing was observed once in eleven observations (Text-fig. 4); in Case 14 (Text-fig. 5) twice in twelve observations; in Case 15 (Text-fig. 6) once in seventeen observations when the fractional pressure was taken by palpation, but not if taken by auscultation; in Case 16 (Text-fig. 7) thirteen times (in two they were equal) in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation.

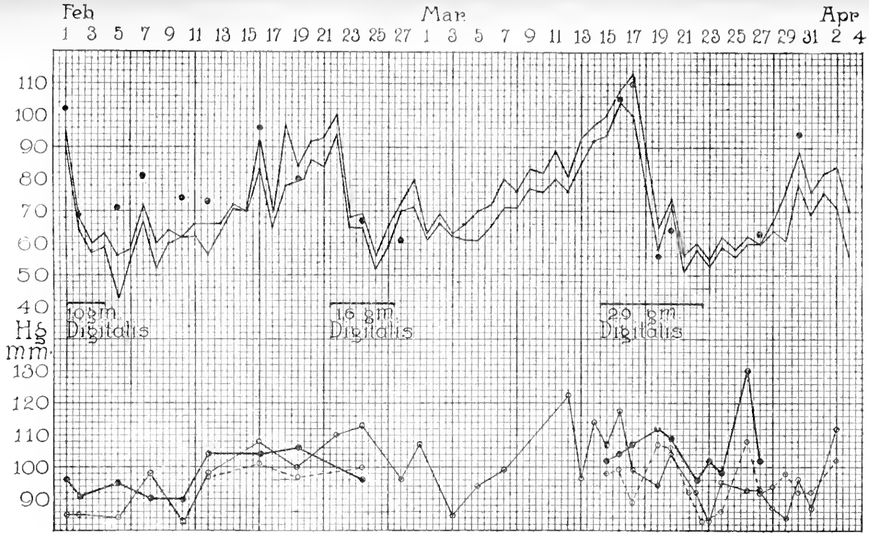
TABLE V.

Case No.	Hospital No.	No. of observations.	Crossing.	
			Observed by method of palpation.	Observed by method of auscultation.
13	3007	11		1
14	2548	12	1	1
15	2683	17	1	
16	2334	19	13	
		20		5

Although the number of patients studied is not large, the fact that in each of them the fractional method of estimating pressure gave readings for the brachial artery lower than for the digital is important. The finding makes it desirable to review the opportunities for error.

An average pressure can no doubt be obtained by the fractional method. But even if the result is obtained free from the technical errors already discussed, and from one other now to be considered, it would probably be incorrect because it is not possible to regard the circulation as is usual in physical phenomena. In order to do this the value of one cycle ought to be directly comparable with another in terms of its numerical equivalent.[6] To regard it in this manner is, however, impossible. If, for instance, a ventricular cycle is unable to lift the aortic valves, this cycle is of no importance in increasing or even in maintaining the pressure in the arterial reservoir. It has no functional value and ought not to be included in calculating the average pressure; it tends merely to lower the value. The only

[6] Kilgore has considered this matter in a similar manner.



TEXT-FIG. 7. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross thirteen times in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation. In the upper set of curves the upper line represents the average ventricular rate for the day, the lower one, the average radial rate. The dots indicate the ventricular rate at the time of blood pressure determination. In the lower set of curves the brachial pressure by the auscultatory method is represented by the heavy line; brachial pressure by the method of palpation is represented by the broken line. Digital pressure taken by the method of Gaertner is represented by the light line.

ventricular cycles which can have an influence on the pressure in the arteries are those the systolic value of which is greater than diastolic pressure. But as far as maintaining the driving force of the circulation is concerned, that is the head of pressure represented by the difference between systolic and diastolic pressure, not all the cycles having a systolic value above diastolic pressure are equal. Many having a low value need have no significance in maintaining the effective pressure. In cases where the rate is low, and where there is no pulse deficit, whether the rhythm is regular or irregular, both a high systolic and a high diastolic level can be maintained. This is observed in cases of complete heart block. Of two such cases studied, the brachial systolic pressure in one was 250, the diastolic 90, and the digital pressure 170; and in the other the systolic pressure was 176, the diastolic 63, and the digital pressure 164. It is apparent that additional ventricular beats, if they are ineffective, whether able to lift the aortic valves or not, would have no significant influence on these figures. The rate in both cases was about 30. A few cycles of great power can therefore maintain a high head of pressure. We infer from this that 30 to 40 beats at a given pressure, 130 mm. for instance, are sufficient to maintain effective pressure close to this level. If additional 40 to 60 beats having low systolic pressure values are introduced into the calculation the numerical value given to the pressure is merely reduced and no added light is thrown on the actual circulatory condition. And if ventricular cycles that do not even develop force sufficient to open the aortic valves are included, the average pressure is still further depressed.

In estimating the systolic pressure in fibrillation of the auricles, therefore, the fractional method is defective. A substitute is readily found in the use of the tonometer method of Gaertner. By its means satisfactory readings are possible. One should remember, however, that the readings so obtained tend to be about 20 mm. lower than the pressure in the brachial artery. As a rough method for estimating the pressure in fibrillation, it appears to us to be sufficient to find rapidly the level of pressure in the brachial artery at which about 40 beats per minute occur; this point is not far removed from the effective pressure about which information is actually required. It avoids, furthermore, the appearance of numerical accuracy, where accuracy, as Mackenzie pointed out, is impossible.

In two patients (Nos. 13 and 16), the subjects of fibrillation of the auricles, we were able to study the effect of digitalis on the brachial and digital pressures. In the first, after giving digitalis for about 8 days, there was a slight lowering of the digital curve (Text-fig. 4) from 97 to 74 mm. But in the period before this, readings of 80 to 85 mm. were recorded and a reading of 84 was found later in the treatment. The influence of digitalis in this instance cannot, therefore, be called striking. In the second case (Text-fig. 7) there were three digitalis periods separated by 14 or more days during which 1, 1.6, and 2.9 gm. were given. In each digitalis period the digital pressure fell, rising again during the intervals between administrations. In the first interval the pressure rose from 83 to 108 mm.; in the second interval from 85 to 122 mm.; in the third interval from 83 to 112 mm. It is the usual experience, when the mechanism of the heart is normal, to find little or no alteration in brachial blood pressure during digitalis administration. Whether this is true when the auricles are fibrillating, is unknown. The fractional blood pressure curve in this patient taken either by palpation or auscultation is unsatisfactory, so that the record of its behavior is not valuable. Nor is it possible to construct a brachial curve based on the levels at which about 40 beats were heard or felt because the counts due to the long examination made necessary by the fractional method are inaccurate. These observations permit us to state merely that we observed not a rise but actually a fall in digital pressure during digitalis administration. The subject requires further study.

SUMMARY.

The function of the arteries as an elastic reservoir between the heart and the capillaries is reviewed. The appropriateness of selecting the exit from this reservoir as the point for estimating its effective pressure is shown. The technique for taking the pressure here by the method of Gaertner is described and its advantage and certain apparent disadvantages are indicated.

The technique of Gaertner is shown to be especially applicable to the study of the blood pressure in fibrillation of the auricles. The use of this technique has brought out a defect in the so called fractional method of taking the pressure in this condition; the brachial and digital curves cross.

Taking pressure of both brachial and digital arteries has shown that certain different types exist; first, that in which both central and peripheral pressures are stable; second, that in which the more central pressure is stable and the peripheral pressure fluctuates; and third, that in which both pressures fluctuate together.

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THE PERIPHERAL BLOOD PRESSURE IN FIBRILLATION OF THE AURICLES.

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PLATE 11.

(Received for publication, January 22, 1918.)

In a study of the blood pressure in persons whose auricles were fibrillating we found that satisfactory readings can be obtained at the digital artery by the method of Gaertner (1). It is well known that the pressure in the brachial artery in auricular fibrillation is difficult to take either by the auscultatory or palpatory method on account of the large differences in pulse pressure of succeeding beats. The method of taking the pressure at the digital artery is more likely to be satisfactory, because the pulse pressure is much smaller at this point and the fluctuations from beat to beat, compared with those in the brachial artery, are probably insignificant.

EXPERIMENTAL.

To determine this point we performed the experiments reported below. Dogs were anesthetized with morphine, paraldehyde, and ether. The right femoral artery was connected with a membrane manometer; the small artery on the dorsum of the left hind foot, with a mercury manometer. Two serrefines, to each of which insulated copper wire was soldered, were fastened to the right auricular appendix. They were introduced into the chest through a small intercostal incision. The other ends of the wires were attached to the secondary coil of an inductorium. The auricles were made to fibrillate by stimulating them with an interrupted current, and the action of the ventricles thereupon became completely irregular.

The curves which we obtained show that when the auricles were in a state of fibrillation, the rate of the ventricles rose, in this case from 123 before the inception of this rhythm to 210 (Fig. 1). The

pulse pressure in the right femoral artery fluctuated through a wide range. But in the artery of the dorsum of the left hind foot the fluctuations in pulse pressure were small and did not vary by more than a few millimeters of mercury. It is clear, therefore, that the pressure here does not vary through so wide a range as in the larger vessels; the value of any one beat differs but slightly from that of the others. In view of the small pulse pressure, the systolic and diastolic limits are not far removed from the mean pressure. The readings are simply and directly made. The experiments show, therefore, that direct readings, not far removed from mean pressure, may be obtained in dogs from the small arteries. In man the pressure conditions in the small arteries, like the digital, are doubtless similar to those in the dog. With a technique such as that recommended by Gaertner, satisfactory estimates of pressure should accordingly be possible. Such estimates have been made and the results obtained are published elsewhere (1).

Our experiments bring out, in addition, certain points bearing on the blood pressure when the auricles fibrillate. In the curve reproduced, the fact appears that the general level of the pressure in the smaller vessel does not fall, but on the contrary tends, on the whole, to rise slightly. A similar rise, probably temporary, was observed in three of the four experiments; the pressure fell in only one. In two of the three instances in which the pressure rose at first, it fell later in the experiment, after prolonged anesthesia and operative manipulation. The behavior of the pressure during experimental auricular fibrillation is of interest to us as it was to Lewis (2), because of the bearing of the experimental data on the phenomena in man. Lewis observed that "the disturbances of the circulation are so profound in the human subject, when this curious disorder of the heart's action begins" that he was impelled on this account to investigate the nature of the new conditions experimentally. We too have seen profound disturbances in the circulation under these conditions, but there has, on the other hand, been abundant opportunity to observe instances in which fibrillation of the auricles set in without subjective sensations in the patient. These cases may find their explanation in those of our experiments in which the general level of pressure and the range of pulse pressure did not change in the smaller vessels. This is im-

portant because the maintenance of the pressure level at this location is one of the functions on which the sufficiency of the circulation ultimately depends. If hearts actually undergo this violent change in mechanism without necessary change in pressure, which our experiments show is possible, the fact that patients undergo the same change without sensation is explicable. That the change is accompanied by severe reaction in many individuals is, of course, well known, and in them, as Lewis and we ourselves found, temporary fall in pressure is easily assumed. The influence of ventricular rate, as Lewis has pointed out, is an important factor under experimental conditions. How important it is within the limits in which it fluctuates in patients we are not in a position to say.

SUMMARY.

1. Experiments on dogs show that whereas the pulse pressure in the larger arteries (femoral) varies extensively, it varies within narrow limits only in the small ones (dorsal artery of the foot).

These results supply an experimental explanation for the fact that in man uniform pressure readings were obtained by Gaertner's method at the digital artery.

2. The experiments show likewise that in certain instances the level of pressure is maintained when fibrillation of the auricles sets in.

It is therefore clear that when the mechanism of the heart beat in man changes to fibrillation of the auricles, a change, that is a fall, in pressure need not necessarily develop.

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EXPLANATION OF PLATE 11.

Fig. 1. The effect of fibrillation of the auricles on the pulse pressure in the right femoral artery and in the dorsal artery of the left hind foot. From above down the figure records the signal of stimulation of the right auricle, the blood pressure of the right femoral artery taken by a membrane manometer, the blood pressure from a dorsal artery of the left hind foot, the time in seconds.

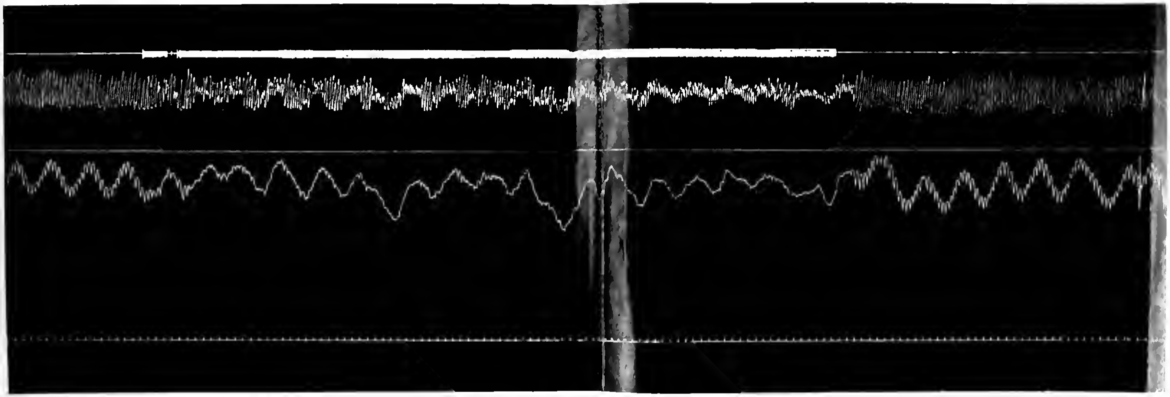

FIG. 1

FREE ANTIGEN AND ANTIBODY CIRCULATING TOGETHER IN LARGE AMOUNTS (HEMAGGLUTININ AND AGGLUTINOGEN IN THE BLOOD OF TRANSFUSED RABBITS).*

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Plates 12 and 13.

(Received for publication, February 28, 1918.)

A singular phenomenon, hitherto undescribed, may frequently be observed in the shed blood of rabbits rendered plethoric by repeated small transfusions from compatible donors.[1] In fresh slide preparations the red corpuscles begin almost at once to clump into masses, and within a few moments the separation of plasma and cells is complete. The blood film, homogeneous at first to the naked eye, is transformed into a mixture of clear fluid and large red flakes. In defibrinated blood allowed to stand at room temperature, the cells fall out rapidly as a red granular sediment, which, in the course of a few hours, may become a solid mass that cannot be broken up without hemolysis. The clumping can occur *intra vasam*, as may be shown by inducing stasis in the rabbit's ear with a tourniquet applied at the base. When the marginal ear vein is opened after ½ hour of such stasis, the blood flowing forth is seen to consist of numerous rather large, dark red flakes in a clear fluid. Under ordinary conditions, the clumping is plainly an extravascular phenomenon, being first

* Read at a joint meeting of the American Association of Immunologists and the Society for Serology and Hematology, New York, April 6, 1917.

[1] The method of transfusion has already been described (Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1917, xxv, 665). The rabbits received intravenously, 6 days in 7, 10 cc. of whole citrated rabbit blood obtained by the cardiac aspiration of compatible donors. For each recipient a series of donors were employed in rotation. Their compatability had been determined by the method of Rous and Turner, *J. Am. Med. Assn.*, 1915, lxiv, 1980.

visible in slide preparations some 12 to 40 seconds after the blood is drawn.

Protection of the Organism.

The clumping of the red cells would result in pulmonary emboli and be quickly fatal, were it not in some way prevented *in vivo*. Our first tests had to do with this phenomenon. The problem presented proved unexpectedly easy of solution. The clumping is absolutely conditioned by temperature. If no precautions are taken to keep warm the rabbit's ear when bound with a tourniquet, clumping occurs in the vessels, as already described. But if the bound ear is kept in water at the body temperature, the blood taken from it after half an hour shows no clumping, and this appears only secondarily as cooling occurs. Again, if a little fresh blood is taken into each of two thick-walled glass tubes of capillary bore, one of which has been chilled in the ice box, the other heated to body temperature, a gross clumping will be seen almost at once in the cold tube, whereas in the warm one the blood remains homogeneous during the 3 or 4 minutes before clotting takes place. Blood allowed to drop directly into a few cubic centimeters of cold salt solution shows clumping almost before it can be distributed in the medium; whereas if the solution has the body temperature, the corpuscles remain separate for 24 hours even—the longest time over which we have observed them. If, after 24 hours of warmth, the mixture is cooled in running water, clumping occurs in case the initial dilution of the blood has not been too great.

The Clumping Is a True Agglutination.

The character of the temperature control suggests that the clumping is caused by hemagglutinins. Landsteiner has shown that the isoagglutinins and the weak normal autoagglutinins of several animal species, among them the rabbit, are similarly governed by temperature and have most effect in the cold.[2] The clumping, viewed microscopically, has certainly the appearance of a true agglutination, while the circumstances of its occurrence are such as would favor the development of agglutinins. It is most pronounced when the rabbit

[2] Landsteiner, K., *Münch. med. Woch.*, 1903, l, 1812.

has received from ten to fifteen transfusions. After the first five or six, a change begins to be noticeable in the shed blood. Rouleau formation is more marked than normally. Then, as the injections are continued, the rouleaux of the shed blood, which are strikingly long and perfect, tend to draw together into masses in which they undergo little disorganization (Figs. 1 and 2). Finally, the tendency to clumping becomes so strong that the rouleaux present when the blood is first shed collapse after a few moments into irregular agglomerates of corpuscles lying against one another without definite arrangement. In slide preparations these masses are at first connected by large trunks of cells, but shrinkage soon takes place—doubtless from closer apposition of the cells—and the trunks pull out into thin strands, often only one or two cells thick (Figs. 3 and 4). Within the large plasma spaces thus opened up, there are almost no corpuscles, red or white. When pressure is put on the cover-glass the cells are often stretched into long ropes, but they hold together tenaciously as if made of a sticky, elastic material. When the pressure is released they resume their normal shape.

If the clumping is caused by a true agglutinin, a separation of this element from the cells should be possible. It has been accomplished by repeatedly washing the cells in warm salt solution in which, as already stated, they are not clumped. Cells thus washed in the centrifuge remain unclumped when sedimented and cooled. But they at once clump when placed in serum obtained from a specimen of the blood defibrinated and centrifuged in the warm.

Attempts were now made to obtain the agglutinin in salt solution by the method Landsteiner employed with the weak, normal autoagglutinin of rabbits.[2] Landsteiner allowed a small quantity of cells in a large quantity of serum to stand over night on ice. Complete agglutination took place. The cell mass was now washed several times in ice cold salt solution and then placed in a little of the fluid at body temperature. After some hours it was centrifuged while still warm. The heating had liberated the agglutinin, which passed into the salt solution, and the latter now possessed the ability to agglutinate cells.

In the case of our transfused rabbits the serum factor responsible for clumping was so strong that there was no necessity for the serum

to preponderate greatly over the cells or for more than a brief chilling and warming.

Experiment 1.—A little of the blood of a transfused rabbit was taken into a test-tube surrounded by a water jacket at 38°C. and was defibrinated with glass beads. From this 0.6 cc. was pipetted off and cooled in ice. The corpuscles rapidly clumped and fell to the bottom of the tube. After 45 minutes centrifugation was done in an ice jacket. The serum was immediately pipetted off and kept. It will be termed Serum A. The cells, which had formed a solid mass, were now twice washed with 3.5 cc. of ice cold salt solution. The cell mass showed no tendency to break up when thus handled. All fluid was now pipetted away, 0.3 cc. of fresh salt solution put on, and the tube transferred to a water bath at 40°C. Within 5 minutes the mass had broken up into a homogeneous cell suspension. After 10 minutes more, an attempt was made to throw down the cells while still warm, but though a warm water jacket was used, the temperature fell sufficiently during the process for some clumping to occur. The tube was therefore warmed again for 15 minutes and again centrifuged rapidly, but now in a jacket of warm paraffin oil. This time the heat was retained, no clumping occurred, and the fluid—Fluid B, as it will be called—was immediately taken off for test. The cell sediment was then twice washed in an excess of warm salt solution and made up to the original blood bulk. The cells remained unclumped.

The following mixtures were now set up:

(*a*) 1 part cell suspension + 3 parts Fluid B.
(*b*) 1 part cell suspension + 3 parts Fluid B + 9 parts salt solution.
(*c*) 1 part cell suspension + 3 parts Serum A + 9 parts salt solution.
(*d*) 1 part cell suspension + 12 parts salt solution.

In (*a*) marked clumping of the cells took place almost at once at room temperature. The other three mixtures were cooled in ice. Strong clumping was observed after a few minutes in (*b*), slight clumping in (*c*), and none at all in (*d*). From the presence of a slight clumping in the mixture (*c*) it is evident that the factor responsible for agglutination was not completely removed from the serum when the defibrinated blood was cooled to 0° C.

Variation with Temperature.

In experiments such as the foregoing, success was obtained only after the necessity for careful maintenance of the essential temperatures had been recognized. During the separation of the agglutinin a moment's accidental cooling or warming was sufficient to fix or liberate it in large part from the cells, thus leading to confusion. When blood was defibrinated in the warm and then gradually cooled in tubes that permitted of microscopic inspection, slight agglutination was

found to appear as the temperature fell from 36° to 35°C. At 33°C. large clumps formed; and at room temperature (22°) the agglutination was massive. When the tube was warmed again, all clumping disappeared at between 35° and 36°C. In the experiment for the separation of agglutinin given in detail above, all agglutinin was not absorbed from the serum at 0°C. This may have been due to insufficient contact of serum and cells owing to the rapid clumping and sedimentation of the latter when suddenly chilled. We have repeatedly noted that a potent serum, if allowed to separate from the clot at room temperature, may contain no agglutinin whatever.

Reversibility of the Reaction.

The rapid variation in the clumping with changes in temperature has led us to investigate the reversibility of the agglutination. A sample of blood was defibrinated in the warm, as usual, filtered through gauze, and placed, first on ice, and, when clumping was complete, in water at body temperature. This was repeated as fast as massive clumping or its reverse, complete dissociation, had occurred. After nine coolings and warmings, the cells still clumped and separated as rapidly and completely as at first. There was an entire absence of the gummy change seen when cells are repeatedly clumped by a heteroagglutinin.[3]

Strength of the Agglutinin.

The great variation in the clumping at different temperatures and the rapidity with which the agglutinating principle is fixed or freed has rendered difficult a precise determination of its strength. We have employed a crude method, allowing the blood to fall from the rabbit's ear, drop by drop, into known quantities of warm salt solution, and noting the agglutination when the mixtures have been cooled for some minutes at room temperature. Under these circumstances the dilution of both antigen and antibody vary, but they vary alike, maintaining practically a constant relation to each other. There is not the same likelihood of error in the ingredients as when mixtures are made of cells and serum separated from each other in the warm.

[3] Landsteiner, K., and Reich, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1905, xxxix, 83

But the temperature of the salt solution must be above 37°C., since even slight cooling results in some clumping of the cells before they can be properly distributed.

The strongest agglutinin found in the transfused rabbits caused well defined clumping in a mixture of one drop of blood with 100 cc. of salt solution; that is, in a plasma dilution of approximately 1: 2,800.[4] No clumping occurred in the 1: 5,600 mixture. The plasma of a second rabbit agglutinated the cells when diluted 500 times. These were exceptional instances. In the majority of cases the plasma failed to clump the cells when it was diluted with more than 20 parts of salt solution.

The clumping phenomenon did not regularly appear in transfused rabbits. Indeed, in ten out of twenty transfused with a special view to its development it was never observed despite the fact that the transfusions were continued far beyond the usual period. Furthermore, in rabbits transfused persistently any agglutinating factor that had developed tended to disappear. A similar disappearance of precipitin following unduly prolonged immunization has been recorded by Tchistovitch[5] and Nuttall.[6] Sudden reductions in the plethora of the recipients, accomplished by bleeding, failed to induce or increase the clumping phenomenon, as did also a use of donors with cells agglutinable by the recipient's plasma.

Agglutination and Anemia.

The peculiar temperature control of the agglutination in the transfused animals has an obvious, if superficial, likeness to that occurring in paroxysmal hemoglobinuria. And the fact that the animals with the strongest agglutinin developed a sudden anemia, in the midst, so

[4] The percentage volume of the blood plasma was reckoned from a comparison of the rabbit's hemoglobin with that of normal rabbits of which the cell plasma ratio had been established with Epstein's hematocrit. In these normal animals the cells averaged 42 per cent and the plasma 58 per cent of the blood volume. Twenty drops of blood were assumed to make 1 cc. This was the case in actual tests.

[5] Tchistovitch, T., *Ann. Inst. Pasteur*, 1899, xiii, 406.

[6] Nuttall, G. H. F., Blood immunity and blood relationship, Cambridge, 1904, 127.

to speak, of their plethora, has in this respect a special interest. The hemoglobin of the rabbit with an agglutinin active in a 1:2,800 serum dilution fell after the fifteenth transfusion from 128 (Sahli) to 75 per cent in the course of 4 days, despite the injection on each of these days of the usual 10 cc. of blood. The transfusions were now stopped, and the hemoglobin fell to 37 per cent in 2 days more, after which a gradual recovery ensued. The animal at no time manifested symptoms of distress. Some of the blood changes in this rabbit and others of like sort have already been described by Robertson in another connection.[7] In these instances the hemagglutinin was at its greatest strength when the anemia developed, while in animals with a weak agglutinin or none, an anemia was never observed, but, on the contrary, plethora was maintained for weeks after the transfusions had been stopped.

No adequate search has yet been made for an hemolysin in the plasma of the animals becoming anemic, but we have chilled, without result, two plethoric rabbits possessing a weak agglutinin (active in a 1:5 dilution of the whole blood) in the hope of initiating a drop in the hemoglobin. The chilling was accomplished by means of ice cold water, in which the well shaved ear of the rabbit was submerged for $\frac{1}{2}$ to 1 hour. Throughout this period the circulation in the cold ear was exceptionally good. The rectal temperature fell to 37°C., considerably below the normal for the rabbit, but not low enough to produce the *in vitro* agglutination of blood corpuscles.

Persistence of the Agglutinin.

The agglutinating principle, once it has appeared in a blood, persists for a long period and is relatively uninfluenced by the disappearance of plethora, or by sudden intercurrent anemia of the sort just described, or by moderate bleedings. In a rabbit, for example, in which the hemoglobin fell from 125 to 27 per cent in the course of a few days, with a gradual return to the normal of 90 per cent, the agglutinin persisted throughout. 110 days after the normal hemoglobin had been finally reached, the blood still showed clumping when di-

[7] Robertson, O. H., *J. Exp. Med.*, 1917, xxvi, 221.

luted with two volumes of salt solution. This was 133 days after the last transfusion.

SUMMARY.

In rabbits transfused almost daily with the whole citrated blood of other rabbits, an extraordinary condition often develops, which manifests itself in an almost immediate clumping together of all the red cells in specimens of the shed blood. This clumping is due to one or more true agglutinins, of which the strength may be such as to cause clumping in a 1: 2,800 plasma dilution.

The agglutinating principle circulates with the corpuscles against which it is effective; but under ordinary circumstances intravascular clumping fails to occur because the union of antigen and antibody can take place only at a temperature several degrees below that of the body. If the temperature is sufficiently lowered, as when a tourniquet is applied to the rabbit's ear, intravascular clumping ensues. In defibrinated blood, gradually cooled, clumping is first noted as the temperature of 35°C. is approached; and at room temperature (22°) the corpuscles will often come together in a short time into a single, solid mass. At 0°C. the agglutination is still more marked. The reaction seems to be completely reversible, for when the blood is warmed again, the clumps break up and disappear at between 35° and 36°C. Cooling and warming with the resultant clumping and dissociation can be carried out many times on the same blood specimen without apparent change in the corpuscles or in the rapidity of the reaction. The response to temperature changes is extremely prompt.

Once it has been elicited, the agglutinating principle may persist for a long time after the transfusions are stopped. In one instance it was still strong 133 days after the last transfusion. During this period the plethora was succeeded by a severe anemia, which in turn was recovered from. In many rabbits no agglutinin develops, and a continuance of the transfusions will not elicit it. Indeed, when present it tends to disappear if the transfusions are persisted in.

In several of the animals in which the agglutinin was strongest, the plethora was suddenly succeeded by severe anemia, despite continued transfusions. The character of the temperature control of

the agglutination, which somewhat resembles that of the hemolysin in paroxysmal hemoglobinuria, has led us to consider whether the blood destruction might not be due to accidental chilling of the animal. Efforts to induce a fall in the hemoglobin by placing the rabbit's ear in ice water have as yet been unsuccessful. Thus far no adequate search for an hemolysin has been made.

The object of the present paper has been to describe a condition in which large amounts of free antigen and antibody circulate together in the organism, and to demonstrate the factor which prevents their union, the results of which could easily be fatal. The causes of the condition will be dealt with in a subsequent communication.

EXPLANATION OF PLATES.

Plate 12.

Fig. 1. A weak clumping phenomenon. The rouleaux are largely intact.

Fig. 2. A weak clumping phenomenon. Marked rouleau formation.

Plate 13.

Figs. 3 and 4. The clumping phenomenon in pronounced form. In the large serum spaces there are almost no free cells.

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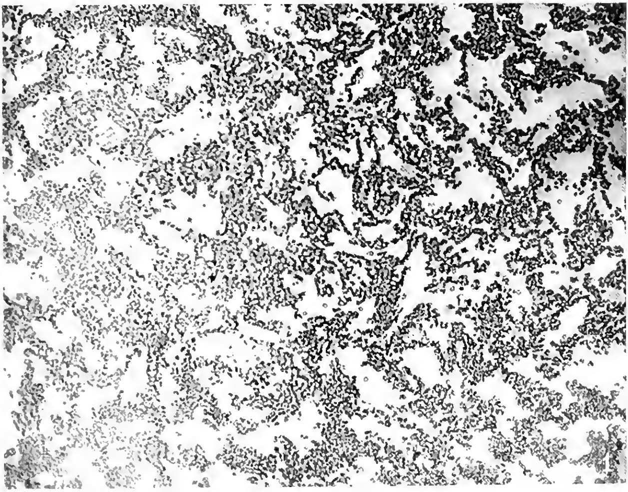
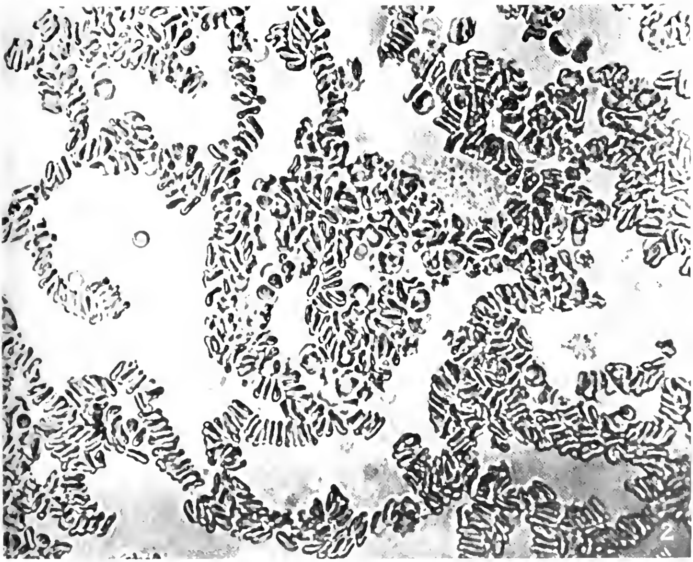
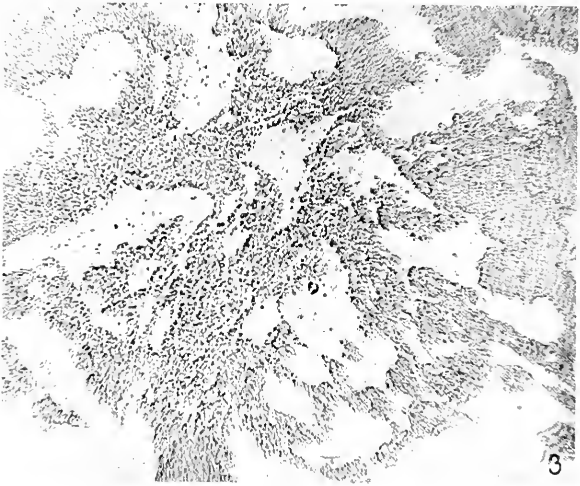
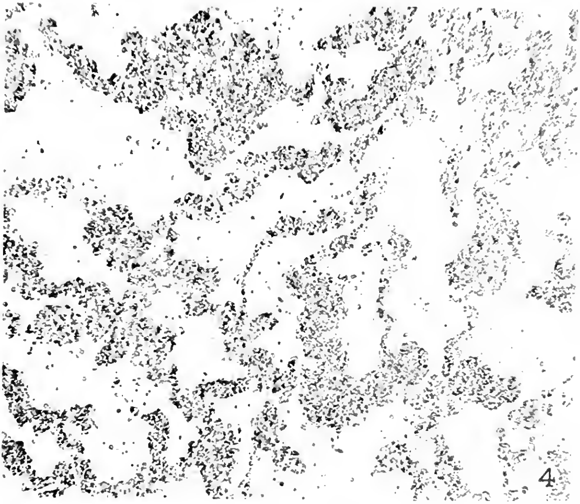




(Rous and Robertson: Free antigen and antibody.)





(Rous and Robertson: Free antigen and antibody.)

A STUDY OF ACUTE MERCURIC CHLORIDE INTOXICATIONS IN THE DOG WITH SPECIAL REFERENCE TO THE KIDNEY INJURY.*

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PLATES 14 TO 16.

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A review of the relatively scant literature dealing with acute mercuric chloride intoxications shows a preponderance of clinical papers with suggestions relative to the treatment of poisoning and bu few investigations which have as their object an understanding of the cause of the remote tissue changes, especially those of the kidney. The investigations which have been primarily concerned with the acute pathology induced by mercuric chloride in organs remote from the intestine have either considered the injury to be dependent upon the action of the metal on the vascular tissues of the organ, or to be due to the action of the metal as such on the parenchyma of the organ during elimination.

Von Mehring[1] considered the toxic action of mercury in the kidney and also in the intes ine to be due to a general vasomotor paralysis. Heineke[2] and Kaufmann[3] held the opinion that mercury in the blood had a thromboplastic action and tha the damage to the kidney depended upon the formation of thrombi with the production of infarcts. Schmiedeberg[4] considers the changes in the

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] von Mehring, J., Ueber die Wirkungen des Quecksilbers auf den thierischen Organismus, *Arch. exp. Path. u. Pharm.*, 1881, xiii, 86.

[2] Heineke, W., Die Fermentintoxication und deren Beziehung zur Sublimat- und Leuchgasvergiftung, *Deutsch. Arch. klin. Med.*, 1888, xlii, 147.

[3] Kaufmann, E., Neuer Beitrag zur Sublimatintoxication nebst Bemerkungen über die Sublimatniere, *Virchows Arch. path. Anat.*, 1889, cxvii, 227.

[4] Schmiedeberg, O., Grundriss der Pharmakologie in Bezug auf Arzneimittellehre und Toxikologie, Leipsic, 5th edition, 1906.

mucous membrane of the colon and kidney to be due to the destructive action of the metal during its elimination. In a recent study of acute mercury poisoning by Burmeister and McNally[5] the same conclusion is apparently reached as that held by Schmiedeberg concerning the way in which the toxic effect of mercury is induced. The authors note the marked variation in the toxic action of the metal and consider that the hepatic changes vary with the duration of the intoxication, while the kidney damage varies with the size of the dose as well as the duration of the intoxication. In the animals receiving massive doses immediate renal changes develop which vary with the size of the dose, while with smaller doses the renal changes depend upon the length of time the animal is able to withstand the intoxication.

In an investigation[6] conducted several years ago, in which various nephrotoxic agents were employed, a study was made of the relative affinity of different kidney poisons for the epithelium of the kidney and of the relation between the degree of epithelial damage and the ability of the kidney to form urine. Mercuric chloride was used in eight animals. The salt was given subcutaneously in the dose of 10 mg. per kilo. The nephropathy induced by these injections was variable in both the frequency with which it occurred and the constancy of the pathological changes in the kidney. Two of the animals became rapidly anuric and showed an extensive necrosis of the renal epithelium, especially that of the convoluted tubules. The remaining animals either showed no toxic effect from the injections, or after a short period of albuminuria made a complete recovery.

Recent studies[7,8] of the acute nephropathy induced in dogs by uranium have shown a similar variation in the toxicity of this metal for the kidney. These studies have furthermore shown that when the functional capacity of the kidney has been reduced by uranium the vascular mechanism retains its responsiveness to various peripherally acting stimuli comparable in degree with that of the normal organ. The lack of functional response has been associated with a variable amount of degeneration of the renal epithelium.

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[5] Burmeister, W. H., and McNally, W. D., Acute mercury poisoning. A parallel histological and chemical study of the renal and hepatic tissue changes as compared with the rapidity of absorption and the amount of mercury present in the circulating blood at the time such changes occur, *J. Med. Research*, 1917, xxxvi, 87.

[6] MacNider, W. deB., A study of the renal epithelium in various types of acute experimental nephritis and of the relation which exists between the epithelial changes and the total output of urine, *J. Med. Research*, 1912, xxi, 79.

[7] MacNider, A study of the action of various diuretics in uranium nephritis, *J. Pharm. and Exp. Therap.*, 1911–12, iii, 423.

[8] MacNider, The vascular response of the kidney in acute uranium nephritis; the influence of the vascular response on diuresis, *J. Pharm. and Exp. Therap.*, 1914–15, vi, 123.

In later papers,[9,10] the observation was made that in acute uranium intoxications the severity of the epithelial damage in the kidney shows a correlation with the degree of acid intoxication induced by the metal, and, furthermore, that the damage to the kidney may in large measure be prevented by the intravenous injection of an alkaline solution.

With these observations in mind the present study of the toxicity of mercuric chloride has been undertaken with the object of ascertaining the cause of the kidney damage and with the hope that some therapeutic agent might be developed that would protect the kidney against the toxic effect of this metal. A preliminary note of this work has recently appeared.[11]

EXPERIMENTAL.

Dogs were used in the experiments which furnish a basis for this study. The animals were placed in metabolism cages, given 500 cc. of water daily by stomach tube, and fed on bread with a small amount of cooked meat. The urine was collected from the cages twice a day. Females were catheterized at the second collection of urine in order to obtain an accurate record of the total output in a 24 hour period. Forty-three of the fifty-six animals used were females. The animals were kept under observation for 3 days prior to administering the mercury. During this normal period (Table I) and following the use of the mercury the animals were studied as follows: Hydrogen ion determinations of the blood were made by the indicator method of Levy, Rowntree, and Marriott.[12] The alkali reserve of the blood and determinations of the carbon dioxide tension of alveolar air were made

[9] MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

[10] MacNider, The efficiency of various diuretics in the acutely nephropathic kidney, protected and unprotected by sodium carbonate. II, *J. Exp. Med.*, 1917, xxvi, 19.

[11] MacNider, A study of the acid-base equilibrium of the blood in acute bichloride intoxications, *Proc. Soc. Exp. Biol. and Med.*, 1917, xiv, 140.

[12] Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen-ion concentration of the blood, *Arch. Int. Med.*, 1915, xvi, 389.

by the methods of Marriott.[13,14] The blood urea determinations were made by the method of Marshall,[15] following the modification suggested by Van Slyke and Cullen.[16] The phenolsulfonephthalein test for kidney function was conducted according to the technique devised by Rowntree and Geraghty.[17] Quantitative albumin determinations were made by Esbach's method, and the percentage of glucose in the urine was estimated by Benedict's reagent. The quantitative determinations of acetone were made by Folin's[18] method as modified by Hart.[19] During the course of the experiments it became necessary to ascertain the relation, if any existed, between the elimination of mercury by the kidney and the development of the acute kidney injury. In the absence of satisfactory quantitative tests the recently devised qualitative test of Elliott was used.[20] The test is both simple and very delicate, and may be employed in a relatively quantitative fashion by noting the amount of mercury deposited on gold leaf as an amalgam from a series of urines.

In order to induce an experimental condition comparable with that obtained when mercuric chloride is taken accidentally or for suicidal purposes, the poison was introduced by a stomach tube. The animals were first given hypodermically 0.25 cc. of a 4 per cent solution of morphine sulfate. After the initial excitement and emesis induced by the morphine, the animals became partially narcotized. During

[13] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[14] Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

[15] Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

[16] Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

[17] Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909–10, i, 579.

[18] Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

[19] Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

[20] Elliott, J. A., A new and delicate method for the detection of mercury, *J. Am. Med. Assn.*, 1917, lxviii, 1693.

this period of narcotization the animals were given 15 mg. of mercuric chloride per kilo. A 1 per cent solution was used. At the time of administration the dose of mercuric chloride was made up to a volume of 100 cc. with distilled water. As a result of the depressed irritability of the animals and the dilution of the dose of the poison, the irritant effect of the mercury was sufficiently modified to prevent vomiting in the majority of the animals for a period of 4 hours. Eight of the animals vomited the mercury within an hour after its use. The rest of the animals retained the poison for 4 hours or longer. Excluding the eight animals referred to above, the poison was unquestionably retained long enough for it to be absorbed and induce its remote toxic effect.

All the animals receiving mercury developed a gastroenteritis which varied widely in severity and duration. This variation apparently depended upon the total amount of the poison received by the animal and the length of time which elapsed between the administration of the poison and the commencement of vomiting. An analysis of the experiments, from the standpoint of the final outcome of the intoxicated animals, permits their classification into four groups. The first group is represented by eight animals (Table II, Group I). The animals of this group developed an intense gastroenteritis which was characterized by persistent vomiting of large quantities of fluid and by frequent, bloody, mucous stools. The animals died in a state of collapse within 48 hours after receiving the poison.

The second group of animals (Table III, Group II) also showed a severe local reaction from the mercury. The vomiting and stools were frequent. In this series the gastroenteritis showed a tendency to subside during the first 3 days of the intoxication. All the animals died within 7 days from the commencement of the intoxication. Six died in convulsions. The remaining four animals died in air-hunger.

The third group of animals (Table IV, Group III) showed a moderately severe gastroenteritis. During the subsidence of the gastroenteritis, or several days after the symptoms of this condition had disappeared, the animals showed a beginning acid intoxication. The hydrogen ion determinations of the blood were variable: The reserve alkali of the blood, however, showed a depletion and the tension of alveolar air carbon dioxide was reduced. These changes, indicative

of a beginning acid intoxication, persisted from 1 to 8 days. The twenty animals forming this group made a complete recovery in as far as any immediate effect from the mercuric chloride intoxication was concerned.

The remaining group of eighteen animals (Table V, Group IV) showed a gastroenteritis which was variable both in severity and duration. In all the animals this symptom of poisoning disappeared. Following the subsidence of the enteritis, and in three of the experiments as late as the 9th day, the animals either gradually or rapidly developed an acid intoxication, and, depending upon the severity and duration of this intoxication, became anuric. Seven of the animals died in air-hunger. From the foregoing outline of the variation in the toxicity of mercuric chloride as shown by the animals comprising the different groups, it becomes necessary to analyze the effect of the poison in the various groups with the object of ascertaining the cause of the variation and the way in which the toxic action of the metal is induced.

Observations on Normal Animals.

The following observations on normal animals extended over a period of 3 days. The results have been recorded in Table I. The number of the experiment in this table corresponds with the number

TABLE I.

Observations on Normal Animals.

Group No	Experiment No.	Urine.	Albumin, glucose, acetone.	Phenolsulfonephthalein.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.
		cc.		*per cent*	*per cent*			*mm.*
I	1	418	0	69	0.012	7.35	8.05	45
I	2	330	0	83	0.021	7.35	8.1	45
I	3	381	0	79	0.013	7.35	8.1	43
II	4	681	0	81	0.014	7.5	8.1	44
II	5	492	0	80	0.012	7.55	8.0	42
II	6	891	0	73	0.012	7.45	8.1	43
III	7	271	0	84	0.013	7.3	8.05	41
III	8	508	0	82	0.012	7.45	8.1	45
III	9	320	0	86	0.012	7.35	8.0	42
IV	10	381	0	71	0.012	7.45	8.1	42

of the animal after it had received the poison. The observations on the various groups of intoxicated animals will be found in Tables II to V. A study of the normal findings contained in Table I shows that all the animals were freely diuretic, and that the urine was free from albumin, glucose, and acetone bodies. The centrifugalized urines did not show casts. The appearance of phenolsulfonephthalein in the urine was not delayed longer than 10 minutes. The total output of the dye in a 2 hour period varied between a minimum output of 69 per cent and a maximum output of 86 per cent. The percentage of blood urea in all the animals remained very constant, varying between 0.012 and 0.021 per cent. The hydrogen ion concentration of the dialyzed whole blood prior to aeration varied between 7.35 and 7.55. After the aeration of the dialysate the reserve alkali showed slight variation, 8 to 8.1. The determinations of the tension of alveolar air carbon dioxide showed a constancy with that of the reserve alkali determinations. In as far as the urine, renal function, and acid-base equilibrium of the animals were investigated, the animals of all the groups were normal.

Observations on the Different Groups of Animals Intoxicated by Mercuric Chloride.

Group I.

The first group of experiments (Table II) is represented by the animals which died from the intoxication within 48 hours after receiving the mercury. All the animals showed a clinical condition comparable with the state of shock and collapse which is obtained in man from the use of a concentrated corrosive poison. They were unable to stand. The surface of the body was cold, the tongue and gums were cyanotic, the respirations shallow, and the heart beat was fast with feeble heart sounds. The pupils in four of the animals were widely dilated for several hours before death. The animals of this group had an intense gastroenteritis, as was indicated by the persistent vomiting of fluid, in two instances streaked with blood, and by the frequent, bloody, mucous stools. The autopsies showed the usual effect of a strong corrosive on the mucous membrane of the stomach and intestine.

TABLE II.

Group I. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools	Vomitus.	Condition of animal
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
1	15	1	790	0	0	0	75	Tr.	0.012	7.45	8.05	42	Severe enteritis. Blood.	Frequent.	Bad.
		2	0	0	0	0	0	0	0.027	7.35	8.0	38	No change.	No change.	Died in collapse.
2	15	1	70	Tr.	Tr.	0	77	Tr	0.040	7.35	8.0	39	Severe enteritis. Blood.	Frequent.	" " "
3	15	1	130	"	0.39	4.612	65	"	0.061	7.3	7.9	37	" " "	"	" " "

A review of Table II, containing representative experiments from this group, shows that the output of urine is rapidly reduced. This reduction is not associated with a comparably great reduction in the elimination of phenolsulfonephthalein. There is no marked retention of blood urea. In Experiment 2, Table II, the animal formed only 70 cc. of urine in the first 24 hour period after receiving the mercury. The elimination of phenolsulfonephthalein was 77 per cent. In Experiment 3 the animal formed 130 cc. of urine in a similar period with a phenolsulfonephthalein elimination of 65 per cent. There is in this group a lack of correlation between the ability of the kidney to form urine and to secrete phenolsulfonephthalein. The urine has been either free from albumin or has contained a mere trace. In only one animal was glucose present in the urine. The urine of this animal also showed a trace of acetone, 4.6120 mg. per 100 cc. In this group of animals only a slight disturbance in the acid-base equilibrium of the blood occurred. In Experiment 2, Table II, at the end of the first 24 hours of the intoxication the alkali reserve was 8 as compared with a normal reading of 8.1, and the tension of alveolar air carbon dioxide was 39 mm. as compared with the normal of 45 mm. The animal died of shock 6 hours later. Experiment 3, Table II, shows the greatest disturbance in the acid-base equilibrium of any of the members of the group. Associated with the reduction in the reserve alkali of the blood the elimination of phenolsulfonephthalein is reduced from a normal of 78 per cent to 65, and both glucose and acetone appear in the urine. At this early stage of the intoxication only a trace of mercury was found in the urine.

The animals comprising Group I die in collapse which is apparently dependent upon the severity of the local corrosive action of the mercury in the stomach and intestine. Death occurs before sufficient time has elapsed for the development of the kidney injury. The reduction in the output of urine is probably dependent upon a disturbance in the functional capacity of the vascular mechanism of the kidney induced by the deflection of arterial blood away from the kidney to the splanchnic viscera. The absence of degenerative changes in the kidney and the presence of an intense congestion of the splanchnic vessels would apparently permit this deduction.

Pathology of the Kidney.—The kidneys of this group of animals

were removed immediately after death. Postmortem changes were eliminated. The kidneys have had a dark cyanotic appearance. On section the organs have shown an engorgement with venous blood which has been especially marked at the corticomedullary boundary zone and has extended into the medulla in the form of streaks outlining the return veins from the venous arches. Histologically the glomerular vessels have shown an engorgement with blood. No exudate or free hemorrhage has been observed in the glomeruli. The endothelial nuclei of the glomerular capillaries have appeared prominent and stained deeply. The epithelium of the tubules, especially that of the convoluted tubules, has shown an early cloudy swelling, consisting in the appearance of albuminous granules in the cytoplasm without much increase in the volume of the cells. The nuclei of the epithelium have shown an increase in size out of proportion to the changes in the size of the cells. No exudate or extravasation of blood has been observed in the intertubular connective tissue. (Fig. 1.)

Group II.

The second group of experiments consists of the animals which first developed a severe gastroenteritis, and then, during the subsidence of the gastroenteritis, developed an acute acid intoxication with an associated anuria. The animals of this group either died in air-hunger or in convulsions.

A review of the experiments representative of this group of animals (Table III) shows that during the 1st day of the intoxication the output of urine was variable. In Experiments 4 and 6 a sharp reduction in the formation of urine occurred, while in the animal of Experiment 5 the output of urine was in excess of the average normal secretion. In all the animals, as the intoxication progressed, the formation of urine rapidly decreased. The amount of albumin in the urine was slight and is no indication of the severity of the kidney damage. Six of the animals of this group showed both glucose and acetone in the urine. The glucose generally appeared in the urine after the appearance of albumin. The appearance of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood. The elimination, however, of these bodies by the kidney does not show a

TABLE III.

Group II. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
4	15	1	312	Tr.	Tr.	14.7321	71	Heavy.	0.016	7.4	8.0	40	Severe enteritis. Blood.	Frequent.	Bad.
		2	418	"	"	4.6371	28	Tr.	0.021	7.3	7.9	32	No change.	Less frequent.	"
		3	121	"	"	3.7241	Tr.	"	0.067	7.2	7.8	20	Less frequent.	Less frequent.	Very bad.
		4	0	0	0	0	0	0	0.078	7.1	7.75	20	No change.	None.	Died in convulsions.
5	15	1	665	0	0	0	35	Tr.	0.015	7.4	7.95	40	Severe enteritis. No blood.	Frequent.	Bad.
		2	31	Tr.	Tr.	Insufficient urine.	0	Insufficient urine.	0.029	7.3	7.85	25	No change.	Occasional.	Very bad.
		4	0	0	0	0	0	0	0.092	7.1	7.75	15	Less frequent. No blood.	None.	Died in air-hunger.
6	15	1	281	0.25	0	0	Tr.	Heavy.	0.033	7.2	7.9	34	Severe enteritis. Blood.	Occasional.	Bad.
		2	31	0.75	Tr.	4.4784	"	Tr.	0.044	7.1	7.55	15	Improved.	None	"
		3	8	1.2	Insufficient urine.	Insufficient urine.	Insufficient urine.	Insufficient urine.	0.081	7.1	7.5	12	No change.	"	Died in air-hunger.

parallel with the reduction in the alkali reserve. With the further depletion of the alkali reserve, and associated with the decreased elimination of phenolsulfonephthalein by the kidney, a decrease in the output of acetone occurs.

The elimination of phenolsulfonephthalein was reduced in all the experiments. The degree of reduction in the output of the dye varied in the different animals. There was no correlation between the quantitative output of phenolsulfonephthalein and the elimination of mercury by the kidney. In Experiment 4 a heavy amalgam of mercury was obtained from the urine of the animal secreted during the 1st day of the experiment, yet the phenolsulfonephthalein output remained high, 71 per cent. In Experiment 5, however, with only a trace of mercury in the urine, the output of phenolsulfonephthalein was reduced from 80 to 35 per cent.

The experiments show a relation between the phenolsulfonephthalein output and the reduction in the alkali reserve of the blood. The animals which showed a rapid depletion in the alkali reserve also showed a sharp decrease in the ability of the kidney to secrete the dye. Following the variation in the output of urine which was noted in the different animals early in the intoxication, as the intoxication progressed all the animals showed a rapid reduction in the formation of urine and became anuric. The anuria in this series of animals was not associated with a condition of shock as in the anuric animals of Group I. With the progressive decrease in the formation of urine the gastroenteritis lessened in severity. Associated with the reduction in the output of urine there occurred a rapid decrease in the elimination of phenolsulfonephthalein, a retention of blood urea, and the development of a severe acid intoxicaton. The animals dying in air-hunger showed the greatest depletion of the alkali reserve of the blood and the most marked decrease in the tension of alveolar air carbon dioxide. In Experiment 5, 2 hours before death the animal had a reserve alkali reading of 7.75 and a tension of alveolar air carbon dioxide of 15 mm. In Experiment 6 the alkali reserve was reduced to 7.5 and the tension of alveolar air carbon dioxide to 12 mm. None of the animals of the group survived longer than 7 days.

Pathology of the Kidney.—The kidneys from this group of animals were either removed immediately after death or the animals were

killed while in air-hunger and the organs secured for the pathological study. The kidneys are pale and swollen. On section the cortex bulges through the cut capsule. The cut surface appears pale and relatively bloodless. The microscopic examination has shown the glomeruli to be in a fair state of preservation. The capillaries are not engorged with blood. The endothelial nuclei are swollen and stain intensely. The epithelium of the tubules, except that of the junctional tubules, has shown a remarkable degree of swelling and early necrosis. The swollen cells in many tubules have not only completely occluded the lumen of the tubule, but give to the tubule an increased transverse diameter. The nuclei are large and hypochromatic. In other tubules the nuclei have disappeared, the cells having undergone complete necrosis. (Fig. 2.)

Group III.

The third group of experiments consists of the animals which received mercuric chloride and, after recovering from the mercury enteritis, developed an acid intoxication and later made a complete recovery. In this group of animals which did not develop the severe gastroenteritis characteristic of the early stage of the intoxication in the two previous groups, the output of urine was not greatly reduced (Table IV). Albumin was present in the urine of all the animals with two exceptions. The amount did not exceed 1 gm. per liter. With the appearance of albumin in the urine the phenolsulfonephthalein output was decreased, a reduction occurred in the alkali reserve of the blood and in the tension of alveolar air carbon dioxide, and acetone bodies appeared in the urine. Usually during the 2nd day these changes in the urine and blood had reached their maximum and from this point in the experiments the animals showed a return to the normal both in regard to the functional capacity of the kidney, and the restoration of the normal acid-base equilibrium of the blood. Since this series of animals went to the stage of recovery, a study of the experiments permits an investigation of the relation between the elimination of mercury by the kidney and the development of the kidney injury. In Experiment 9, Table IV, on the 1st day of the intoxication a heavy precipitate of mercury was obtained from the urine. The amount of urine formed showed an increase over the

TABLE IV.

Group III. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		cc	gm.	per cent	mg.	per cent		per cent			mm.			
7	15	1	621	0	0	0	85	Heavy Tr.	0.012	7.3	8.0	38	Enteritis. No blood.	None.	Good.
		2	297	0	0	5.4114	85	Tr.	0.012	7.4	7.95	34	" improved.	"	Improved.
		4	343	0	0	5.1315	84	"	0.012	7.45	8.05	40	Normal.	"	Appears normal.
		10	497	0	0	11 6625	87	0	0.012	7.55	8.1	45	"	"	Recovery.
8	15	1	655	0.5	0	7.5321	78	Heavy Tr.	0 019	7.4	7.95	38	Severe enteritis. Blood.	Occasional.	Bad.
		2	283	1.0	2.15	12.4210	66	Amount decreased.	0.019	7.4	7.95	38	Improved.	None.	Improved.
		4	450	Tr.	0.2	18.3241	80	0	0.015	7.5	8.1	45	Normal.	"	"
		8	670	0	0	17.6714	82	0	0.015	7.5	8.1	45	"	"	Recovery.
9	15	1	750	0	0	2.5657	85	Heavy Tr.	0.015	7.45	8.0	42	Severe enteritis. No blood.	Occasional.	Good.
		2	580	Tr.	0	6.1885	75	" "	0.026	7.4	7.9	35	Enteritis improved.	"	Improved.
		3	623	"	Tr.	6.2431	83	Amount greatly increased.	0.022	7.5	8.0	40	Improved.	None.	"
		6	220	"	"	11.8957	86	0	0.015	7.45	8.05	40	"	"	"
		10	481	0	0	4.7382	85	0	0.013	7.45	8.05	40	Normal.	"	Normal.
		12	531	0	0	0	85	0	0.013	7.45	8.05	40	"	"	Recovery.

normal, the elimination of phenolsulfonephthalein showed practically no reduction, 86 to 85 per cent, and the reserve alkali of the blood remained unchanged. On the 2nd day of the experiment the elimination of mercury was still heavy, while the formation of urine remained nearly normal. The phenolsulfonephthalein output was reduced only 10 per cent. The alkali reserve of the blood was reduced from 8 to 7.9. On the 3rd day of the experiment the elimination of mercury was greatly increased. There was, however, an increased formation of urine, 623 cc., the output of phenolsulfonephthalein had returned to 83 per cent, the normal output being 86 per cent, and the alkali reserve of the blood had returned to the normal reading. Experiments 7 and 8 also illustrate the lack of relation between the elimination of mercury by the kidney and the decrease in the functional capacity of the organ.

Group IV.

The animals comprising this group developed a moderately severe mercury enteritis. During the enteritis, or following its subsidence, the animals developed an acid intoxication which varied in degree and duration. In this respect this group of animals resembles the experiments of Group III. The animals of Group III, however, gradually returned to the normal and made a complete recovery, while the animals of Group IV, after a period during which there was an attempt to restore the normal acid-base equilibrium, became severely intoxicated and showed a more marked depletion of the alkali reserve than was obtained in the initial acid intoxication. The formation of urine was rapidly reduced. The animals were anuric from 1 to 6 days before death.

A study of Table V, which gives in detail the results obtained in one of the experiments of this group, shows that the secretion of urine was rapidly reduced. Associated with the reduction in the output of urine the animals became albuminuric, and later both glucose and acetone appeared in the urine. The amount of albumin was small, not over 0.9 gm. per liter. The glycosuria rapidly disappeared. The output of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood but did not show quantitatively

TABLE V.

Group IV. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
10	15	1	870	0.4	Tr.	0	63	Tr.	0.015	7.3	8.05	38	Severe enteritis.	Frequent.	Bad.
		2	344	0.9	0.38	7.9304	47	F. Tr.	0.055	7.2	7.9	36	Enteritis improved.	Occasional.	"
		3	106	0.5	1.1	9.7965	13	Very F. Tr.	0.060	7.25	7.85	33	Improved.	None.	Improved.
		4	277	0.2	Tr.	4.1985	26	" " "	0.068	7.3	7.95	36	Normal.	"	"
		5	178	F. Tr.	"	10.2130	41	0	0.062	7.4	7.95	35	"	"	No change.
		6	232	0	0	14.162	50	0	0.018	7.4	8.0	38	"	"	Improved.
		10	387	0	0	14.7631	51	0	0.029	7.4	7.95	38	"	"	No change.
		12	81	0	0	7.3620	13	0	0.072	7.15	7.75	23	"	"	Bad.
		13	0	0	0	0	0	0	0.092	7.1	7.7	20	None.	"	Died in air-hunger.

a parallel with the decrease in the alkali reserve. As the acid intoxication progressed the eliminaton of acetone bodies was diminished. The decrease in the elimination of phenolsulfonephthalein is not proportionate to the decreased formation of urine. In this group of animals, as in the other groups, there is more nearly a correlation between the degree of acid intoxication and the elimination of the dye than there is between the output of urine and its elimination. Associated with the reduction in the output of phenolsulfonephthalein a retention of blood urea occurs. The stage of improvement in this group of animals was characterized by an increased formation of urine, a decrease and final disappearance of albumin from the urine, an increase in the output of acetone bodies and phenolsulfonephthalein, a decrease in the percentage of blood urea, and a return towards the normal acid-base equilibrium of the blood. None of these conditions reached the point of normality. From the 6th to the 10th day in Experiment 10, the urine was free from albumin, the phenolsulfonephthalein elimination was 48 to 51 per cent, and the reserve alkali 7.9 to 7.95. On the 11th day the terminal acid intoxication commenced. The following day only 81 cc. of urine were formed. The urine was free from albumin. The phenolsulfonephthalein elimination had been reduced from 51 to 13 per cent and the alkali reserve was reduced from 7.95 to 7.75. The animal was anuric the following day and died in air-hunger.

A study of the elimination of mercury by the animals of this group is of special interest. During the entire course of Experiment 10, Table V, only a trace of mercury was found in the urine. After the 4th day of the intoxication the urine was free from mercury. As will be seen by referring to the table, the kidney damage which was associated with the death of the animal did not occur until the 11th day of the experiment, 7 days after the urine had been free from mercury.

Pathology of the Kidney.—The kidneys of this group of animals have been pale and relatively bloodless. Extending from the corticomedullary junction into the cortex are streaks of fatty degeneration. The histological examination has shown the glomeruli shrunken and not infrequently surrounded by an exudate of serum, fibrin, and red cells. The endothelium of the glomerular capillaries has shown an

advanced degeneration. The nuclei have shown fragmentation. The loops of capillaries are fused together. The epithelium of the tubules has shown an advanced necrosis. The structure of the cells is completely lost. The tubules are outlined by a structureless mass of necrotic material. The tubules have frequently been separated by an exudate containing red cells and fibrin. (Fig. 3.)

SUMMARY.

A study of the experiments comprising the first group of animals permits the deduction that these animals succumb to the acute poisoning as a result of the shock which the poison induces through its corrosive action in the stomach and intestine. The animals die before the mercury, acting as such during its elimination by the kidney, can induce an acute nephropathy and before the mercury, by inducing an acid intoxication, can lead to an acute kidney injury.

The remaining animals of the series, Groups II, III, and IV, have withstood the corrosive action of the poison. These animals have shown the same type of delayed intoxication from the poison. The intoxication, however, has varied in time of appearance, duration, and severity..

The animals classified as Group II have developed during the stage of improvement from the gastroenteritis a rapid and severe type of acid intoxication, have become rapidly anuric, and have died either in a state of air-hunger or in convulsions.

The animals of Group III, either during or after their recovery from the gastroenteritis, have developed a mild grade of acid intoxication. During the following days of the experiments the animals succeeded in reestablishing their normal acid-base equilibrium. All the animals of this group recovered.

The animals of Group IV have shown a recovery from the mercury enteritis. Following a period during which there was an attempt on the part of the animals to return to normal, as indicated by an increase in the alkali reserve of the blood and by an increased output of phenolsulfonephthalein and urine, the members of the group developed a delayed acid intoxication, and, like the animals of Group II, became anuric.

The animals of all groups which have died from the delayed intoxication caused by the mercury have shown a severe type of kidney injury which has been characterized by an acute swelling and necrosis of the renal epithelium. All these animals have either gradually or acutely developed a severe type of acid intoxication. There has been a definite association between the development of an acid intoxication and the delayed kidney injury, and, furthermore, the animals which have shown the greatest swelling and necrosis of the renal epithelium have also shown the severest type of intoxication.

CONCLUSIONS.

1. In the acute mercuric chloride intoxications which have been induced in dogs death has been due either to the shock associated with the severe mercury enteritis or to a delayed kidney injury.

2. The injury to the kidney has been constantly associated with the development of an acid intoxication.

3. The delayed kidney injury is not due to the action of the mercury as such during its elimination by this organ.

4. The manner in which mercuric chloride induces an acid intoxication is at present under investigation. The participation of the liver in the intoxication will be considered in this connection.

EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 3, Table II. The glomerulus, *a*, appears normal except for the deeply staining endothelial nuclei. The epithelium of the convoluted tubules, *b*, shows the presence of albuminous granules with little increase in the size of the epithelial cells. The nuclei stain well. The convoluted tubule epithelium at *c* shows an early swelling of the cells. The animal died in collapse associated with the corrosive action of mercuric chloride.

PLATE 15.

FIG. 2. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of Experiment 6, Table III. It shows at *a* the histologically well preserved glomerular capillaries. The endothelial nuclei are swollen and stain intensely. At *b* is shown the severe swelling of the convoluted tubule epithelium

which has occluded the lumen of the tubule and increased the size of the tubule. The nuclei of these cells are large and hypochromatic. At *c* are shown tubules that have become completely necrotic. The junctional tubules, *d*, do not show the severe swelling and necrosis which has developed in the more highly specialized tubular epithelium. The animal became anuric from the mercuric chloride intoxication and died in air-hunger.

Plate 16.

Fig. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 10, Table V. It shows at *a* the glomerulus with fused capillary loops containing endothelial nuclei which have undergone fragmentation. The convoluted tubules at *b* have become completely necrotic. The outline of the tubule is formed of a structureless, necrotic mass. At *c* an exudate is shown separating the tubules, which consists of serum, fibrin, and red blood cells. The figure illustrates the changes in the kidney of the animals of Group IV, Table V, which recover from the mercury enteritis and later succumb from the delayed intoxication which is associated with the development of an acid intoxication.

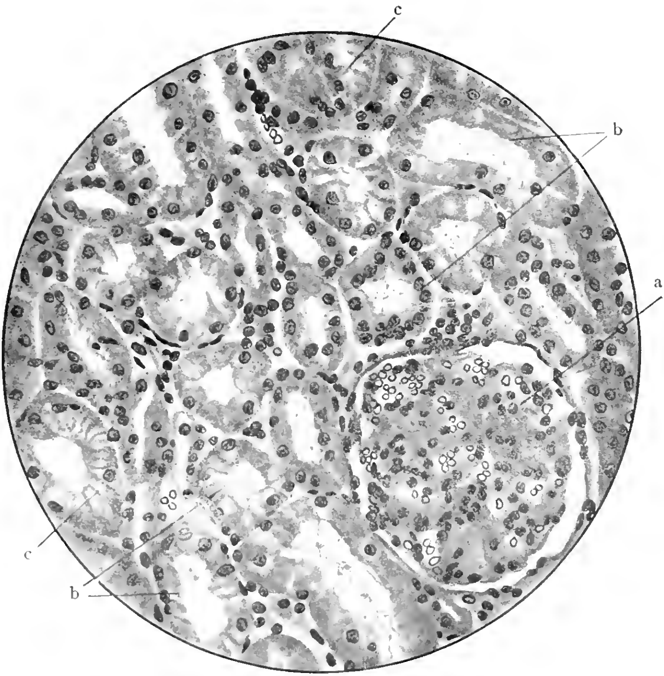

FIG. 1.

(MacNider: Mercuric chloride intoxications.)

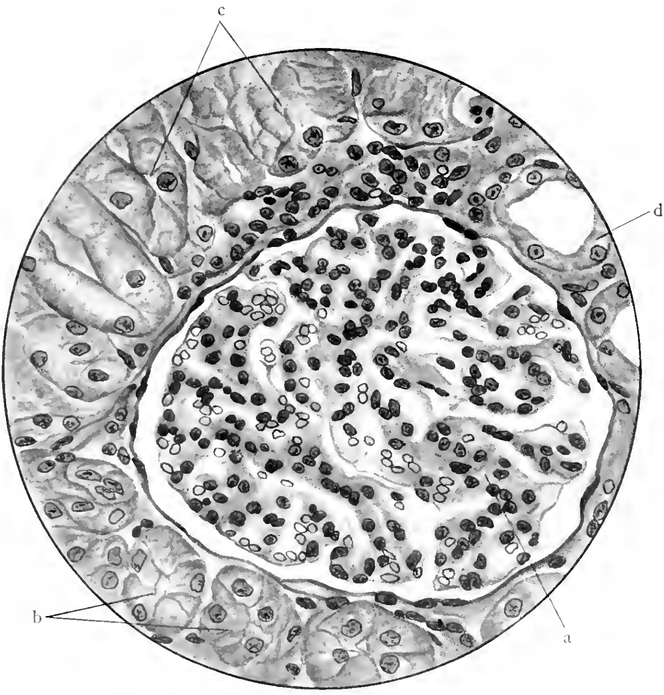

FIG. 2.

(MacNider: Mercuric chloride intoxications.)

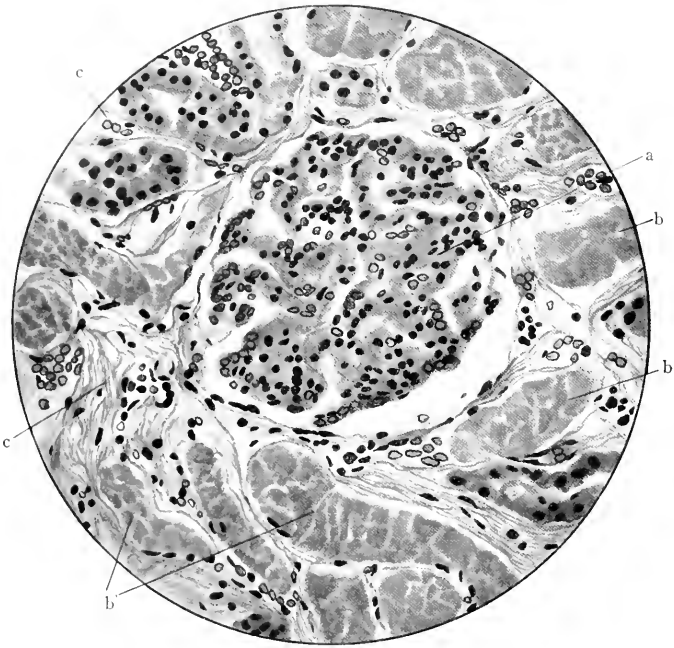

FIG. 3.

(MacNider: Mercuric chloride intoxications.)

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Theodore Caldwell Janeway

Born 1872 Died 1917

At a meeting of the Board of Scientific Directors of The Rockefeller Institute for Medical Research, held on January 19, 1918, the following Minute was adopted:

RESOLVED, *That the Scientific Directors of The Rockefeller Institute record their profound sense of loss in the death of their honored and beloved associate,* THEODORE CALDWELL JANEWAY, M.D., *who has served on the Board with devoted zeal since his election to succeed Dr. Christian A. Herter in 1911. Dr. Janeway at the height of his powers and in the midst of the most productive period of his life was stricken with pneumonia while in active service in the Medical Corps of the Army, to which, since the United States entered into war with Germany, he gave invaluable and unmeasured service. His life was sacrificed to patriotic duty rendered to his country without reserve. Dr. Janeway's period of office on the Board of Scientific Directors of The Rockefeller Institute was restricted to a brief seven years, yet its importance was very great, as he brought to its service learning, keen intelligence, and broad vision.*

Dr. Janeway was a highly skilled and widely read clinician, and he was also a notable exponent of the scientific method in internal medicine. A graduate of the Sheffield Scientific School and of the College of Physicians and Surgeons, he emphasized the importance of chemistry and physics, the two sciences on which he based his clinical conceptions. Coming early under the mature and wise influence of his distinguished father, he received from him the more purely clinical and pathological impress which so much contributed to his broader development. In rapid succession Dr. Janeway became instructor in medicine at New York University and Bellevue Hospital Medical College in 1905, associate professor and then professor of medicine at Columbia University in 1909. During this period, in 1907, he was instrumental in founding the Russell Sage Institute of Pathology, which throughout its connection with the City Hospital was made a valuable adjunct to the courses in medicine which he conducted. It was natural and logical, because of the work he had done in internal medicine, that Dr. Janeway should be called to fill the full time chair in internal medicine established at the Johns Hopkins Medical School in 1914. The acceptance of the new professorship was made at a large financial sacrifice, but his altruistic action was wholly consonant with the broad and sympathetic attitude which he always held toward medical teaching and research.

Dr. Janeway's untimely death cut short not only a career in medicine which he had inaugurated with every promise of distinguished success, but has at the same time deprived The Rockefeller Institute of one of its ablest and wisest counsellors, and the medical profession of a great physician.

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A STUDY OF THE LOW BLOOD PRESSURES ASSOCIATED WITH ANAPHYLACTIC AND PEPTONE SHOCK AND EXPERIMENTAL FAT EMBOLISM, WITH SPECIAL REFERENCE TO SURGICAL SHOCK.

By J. P. SIMONDS, M.D.

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Plates 17 to 21.

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In a preliminary report[1] attention was called to certain fundamental differences between the low blood pressures associated with peptone shock and experimental fat embolism. Consideration of these differences is important because they have a direct bearing upon certain theories as to the etiology and mechanism of surgical shock. It is proposed in this paper to give in detail the experimental data upon which some of the statements made in that report were based, to add new observations, and to discuss the results obtained. The theories concerned are that surgical shock is due to loss of vascular tone in the splanchnic region, that it is due to loss of peripheral vascular tone, and that it is due to fat embolism.

Mann[2] is of the opinion that "the cause of shock is an actual loss of red cells and fluid from the circulating blood through stasis, diapedesis, exudate, and endothelial changes brought about by the reaction of the great delicate splanchnic area to irritation." Janeway and Jackson[3] concluded from their study of low blood pressures induced by compression of the inferior vena cava, that the dilatation of the peripheral venules and capillaries as a result of the increased venous pressure caused such a loss of tone, that even after normal pressure was restored by release of the compression, they became overfilled with blood. As a

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[1] Simonds, J. P., *J. Am. Med. Assn.*, 1917, lxix, 883.

[2] Mann, F. C., *Bull. Johns Hopkins Hosp.*, 1914, xxv, 205.

[3] Janeway, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1914–15, xii, 83. Janeway, H. H., and Jackson, H. C., *ibid.*, 1914–15, xii, 193.

result of this, the arterial pressure fell to a very low level and the animal died in a few hours. Warthin,[4] Bissell,[5] and Porter[6] are convinced that fat embolism is "a cause" of surgical shock.

Technique.

All the experiments here reported were made upon dogs under ether anesthesia. Altogether, more than thirty-five animals have been used in the study of the problems involved. The arterial pressure was taken from the carotid artery according to the usual technique. The fat, in the form of neutral olive oil, was injected through a cannula in the femoral vein, being washed into the vessel with 5 or 6 cc. of isotonic salt solution. Witte's peptone (0.5 to 1 gm., according to the size of the dog) was dissolved in 10 cc. of salt solution and injected through the same cannula. Standard doses of nicotine (1 cc. of a 1:4,000 solution) and of adrenalin (1 cc. of a 1:50,000 solution) were also administered intravenously in the same manner.

In making a record of the venous pressure the following method was employed. The external jugular vein was exposed as low down in the neck as possible. Into it a wide cannula with a large bulb completely filled with 10 per cent solution of sodium carbonate was inserted. The long proximal end of the cannula, measuring 4 to 6 cm., was pushed well down into the subclavian vein and even in some instances into the superior vena cava. Before inserting the cannula into the vessel, a rubber tube was attached to the distal end and closed with a screw clamp to prevent the carbonate solution from running into the vein. The cannula was then connected by rubber and glass tubing (including a T-tube) with a manometer of the type described by Hoskins and Gunning.[7] All connections were made airtight, because air transmission was used. In the manometer there was a solution of zinc chloride with a specific gravity of 1.36; that is, one-tenth the specific gravity of mercury.[8] The float in the manom-

[4] Warthin, A. S., *Internat. Clinics*, 1913, iv, series 23, 171.

[5] Bissell, W. W., *Surg., Gynec. and Obst.*, 1917, xxv, 8.

[6] Porter, W. T., *Boston Med. and Surg. J.*, 1917, clxxvi, 248.

[7] Hoskins, R. G., and Gunning, R. E. L., *Am. J. Physiol.*, 1917, xliii, 298.

[8] For the preparation and the accurate measurement of the specific gravity of this solution I am indebted to Professor J. H. Long.

eter was attached by a thread to the longer branch of a light heart lever exactly half way between the fulcrum and the writing point. After all the connections had been made, it was necessary to produce a slight negative pressure in the apparatus. This was readily done through the T-tube. The screw clamp at the cannula was then cautiously released. The apparatus was so adjusted that the column of liquid forced out of the cannula in high venous pressures did not rise above the level of the vein. In other words, the column of liquid was kept parallel with the axis of the vessel. The respiratory and other changes in the venous pressure were recorded as shown in Figs. 1, 2, and 3. The change in position of the writing point of the heart lever represented ten times the change in pressure expressed in millimeters of mercury, because the fluid in the manometer had a specific gravity one-tenth that of mercury.

EXPERIMENTAL.

The three following protocols of experiments are typical. All operative procedures were carried out under ether anesthesia, and the animal was killed at the end of the experiment without recovery from the anesthetic. A cannula was placed in the trachea and connected with an ether bottle. The remainder of the technique has been sufficiently described above.

Dog 1.—Male; weight 18 pounds. October 15, 1917.
10.07 a.m. Blood pressure 130 mm. of mercury.
10.07 to 10.09 a.m. 12 cc. of olive oil injected in doses of 2 cc. each.
10.10 a.m. Blood pressure 110 mm.
10.13 a.m. Blood pressure 130 mm.
10.13 to 10.15 a.m. 8 cc. of olive oil injected in doses of 2 cc. each.
10.14 a.m. Blood pressure 125 mm.
10.16 a.m. Blood pressure 105 mm.
10.20 a.m. Blood pressure 95 mm. 4 cc. of olive oil injected.
10.21 a.m. Blood pressure 80 mm.
10.25 a.m. Blood pressure 70 mm. 2 cc. of olive oil injected.
10.26 a.m. Blood pressure 65 mm. 2 cc. of olive oil injected.
10.27 a.m. Blood pressure 50 mm. Respiration ceased. Artificial respiration started. Heart stopped beating at 10.29 a.m.

Autopsy.—Dilatation of the right side of the heart; left side contains small amount of blood. General venous stasis. Very slight edema of the lungs.

Dog 2.—Male; weight 14 pounds. June 29, 1917.

1.20 p.m. Blood pressure 180 mm. of mercury. 1 gm. of Witte's peptone injected intravenously. Blood pressure fell within 30 seconds to 55 mm.

1.40 p.m. Blood pressure 130 mm.

1.50 p.m. Blood pressure 170 mm.

1.50 to 1.52 p.m. Three injections of olive oil, 2 cc. each.

1.53 p.m. Blood pressure 170 mm. 2 cc. of olive oil injected. Respiration became slow and shallow and blood pressure began to decline very slowly.

1.57 p.m. Blood pressure 120 mm. Respiration continued to become slower and slower. Blood pressure fluctuated but had a general downward tendency. Reaction to adrenalin and nicotine normal.

2.25 p.m. Blood pressure 70 mm. Respiration slow and labored.

2.40 p.m. Blood pressure 85 mm.

2.55 p.m. Respiration stopped entirely. Blood pressure 50 mm. Artificial respiration begun and ether removed. Blood pressure steadily rose during the artificial respiration until 3.05 p.m.

3.05 p.m. Blood pressure 95 mm. Respiration became spontaneous and artificial respiration was stopped. Immediately upon stoppage of artificial respiration the pressure rose rapidly.

3.10 p.m. Blood pressure 140 mm. Ether given again because of return of corneal reflex. Respiration again became slow and labored. During the next 50 minutes the blood pressure fluctuated between 140 and 70 mm.

4.05 p.m. Blood pressure 90 mm. 5 cc. of olive oil injected. Blood pressure fell to 80 mm.

4.06 p.m. Blood pressure 90 mm. 5 cc. of olive oil injected. Blood pressure fell to 75 mm.

4.07 p.m. Blood pressure 80 mm. 3 cc. of olive oil injected. Blood pressure fell to 70 mm.

4.08 p.m. Blood pressure 70 mm.

4.09 p.m. Blood pressure 60 mm.

4.10 p.m. Blood pressure 55 mm. Respirations very slow and shallow.

4.11 p.m. Blood pressure 40 mm. Respiration stopped.

4.13 p.m. Heart stopped.

Dog 3.—Male; weight 42 pounds. October 18, 1917.

11.33 a.m. Blood pressure 130 mm.

11.36 a.m. Blood pressure 125 mm. 1 gm. of Witte's peptone injected.

11.37 a.m. Blood pressure 55 mm. Venous pressure fell during this time approximately 14 mm.

11.47 a.m. Blood pressure 120 mm. Venous pressure at its former level.

11.47 a.m. to 12.08 p.m. 40 cc. of olive oil injected in doses of 5 cc. each.

12.08 p.m. Blood pressure 110 mm. The reactions of the venous and arterial pressures to the injections of oil are shown graphically in Fig. 3.

12.10 to 12.11 p.m. 10 cc. of olive oil injected in doses of 5 cc. each.

12.12 p.m. Blood pressure 105 to 110 mm.

12.13 p.m. 5 cc. of olive oil injected.

12.14 p.m. Blood pressure 95 to 100 mm. The pressure steadily declined and heart stopped beating at 12.16 p.m. During the fall of arterial pressure the venous pressure constantly rose (Fig. 3).

Autopsy.—Dilatation of the right side of the heart; left side contains very little blood. General venous stasis. Slight edema of the lungs.

The characteristic feature of anaphylactic and peptone shock in the dog is a marked and abrupt fall in arterial blood pressure. Experiments by Edmunds,[9] Manwaring,[10] Denecke,[11] Jaffé and Přibram,[12] and Weil[13] have shown that the liver is an essential element in the production of this type of shock in this animal[14] (Figs. 1 and 4). Although Robinson and Auer,[15] by means of the electrocardiograph, found disturbances in the conduction of the heart and abnormalities in the ventricular contractions, it is evident that the action upon the heart is of relatively minor importance. The cause of the low blood pressure is a stagnation of the blood in the liver and splanchnic region which prevents the right heart from receiving sufficient blood to keep the left ventricle supplied with an amount adequate to maintain the arterial pressure at its normal level.[16]

The studies of blood pressures in experimental fat embolism reported in the literature are meager and for the most part give too few details to be satisfactory. Warthin[4] injected 7 cc. of olive oil directly into the heart of a dog, and observed a rapid fall in arterial pressure and an increase in pressure in the auricle and jugular vein, "as shown in the charts." But no "charts" appear in the article. He states further that "repeated injections cause large systolic pulsations in the right auricle, the arterial pressure steadily falls, and that in the auricle and jugular steadily goes up. Finally there is delirium cordis and death." The weights of

[9] Edmunds, C. W., *Z. Immunitätsforsch., Orig.*, 1913, xvii, 105; 1914, xxii, 181.

[10] Manwaring, W. H., *Z. Immunitätsforsch., Orig.*, 1911, viii, 1.

[11] Denecke, G., *Z. Immunitätsforsch., Orig.*, 1913–14, xx, 501.

[12] Jaffé, R. H., and Přibram, E., *Virchows Arch. path. Anat.*, 1915, ccxx, 213.

[13] Weil, R., *J. Immunol.*, 1917, ii, 525.

[14] Loewit, M., (*Arch. exp. Path. u. Pharm.*, 1913, lxxiii, 1) has pointed out certain alleged differences between anaphylactic and peptone shock.

[15] Robinson, G. C., and Auer, J., *J. Exp. Med.*, 1913, xviii, 556.

[16] Eisenbrey, A. B., and Pearce, R. M., *J. Pharm. and Exp. Therap.*, 1912–13, iv, 21.

the animals, the quantities of oil used in the "repeated injections," and the time allowed to elapse between them are not stated.

Bissell[5] gives one chart showing the arterial and venous pressures in a dog weighing 7.4 kilos (16.3 pounds) which received 17 cc. of olive oil in doses of 7, 5, and 5 cc. within 2 minutes. After the first injection (of 7 cc.), there was only a "volume change" in arterial and venous pressure; after the second, there was a very transient fall in arterial pressure and an equally transient rise in venous pressure; after the third injection, the venous pressure went up very abruptly while the arterial pressure gradually fell and the animal quickly died.

Porter[6] mentions experiments upon eight cats. One received 3 cc. of "officinal emulsion" of olive oil injected slowly into the jugular vein. Very soon there followed a fall in carotid pressure. In two other experiments thick cream was used, and in the remainder, olive oil. "2 to 4 cc. of olive oil in a large cat has never failed to produce a fall in blood pressure to one-half or less the normal level." In one experiment the diastolic pressure fell from 140 to 65 mm. of mercury and later to 40 mm. "In this cat the tracing showed that the fall in blood pressure could not be ascribed to changes in the heart beat. The same is usually true when the injection is not made too rapidly. The clinical picture is essentially that of traumatic shock in human beings."

It appears that in the above instances cited from the literature relatively large amounts of oil in proportion to the body weight of the animal were injected within the space of a few minutes. Graham,[17] on the other hand, made repeated small injections of small amounts of olive oil into the veins of rabbits over a period of days, but no blood pressure tracings were taken.

The presence of fat embolism in some cases of surgical shock has been established by the work of Bissell.[5] But it has also been found by Flournoy,[18] and by Katase,[19] in a variety of other pathological conditions.

In experimental fat embolism, the fall in blood pressure is gradual and progressive. But the beginning of the fall in pressure is not synchronous with the beginning of the injections of the oil. A remarkably large amount of oil can be introduced into the veins of an anesthetized dog without producing more than a very slight and temporary fall in arterial pressure. There appears to be a roughly quantitative relation between the body weight of the dog and the amount of olive oil that must be injected to cause the arterial pressure to start downward permanently. In the experiments of this series it was

[17] Graham, G. S., *J. Med. Research*, 1907, xi, 459.

[18] Flournoy, T., Contribution à l'étude de l'embolie graisseuse, Inaugural dissertation, Strassburg, 1878, quoted by Warthin.[4]

[19] Katase, A., *Cor.-Bl. schweiz. Aertze*, 1917, xlvii, 545.

necessary to inject approximately 1 cc. of oil for each pound of body weight in order to bring about a fall of pressure of any duration. The results in twelve animals are shown in tabular form in Table I. In this table are shown the weight of the animal and the number of cubic centimeters of oil that were injected before the arterial pressure was permanently and materially lowered. It was somewhat difficult to establish a standard. But for the purposes of this tabulation, it was considered that the pressure had been permanently and materially reduced when there had been a lasting fall of 20 to 30 mm. of mercury from the initial pressure, provided the decline did not reduce the pressure below 100 mm. of mercury. The minimum was 29 cc. for a 35 pound dog; the maximum, 70 cc. for a 30 pound dog. If we omit from consideration Dog 13, which was distinctly abnormal in his resistance to the injections of oil, it is seen that the average quantity of oil necessary to initiate a permanent fall in blood pressure in the dog is approximately equal to 1 cc. of oil for each pound of weight of the

TABLE I.

The Quantity of Oil Necessary to Reduce Permanently and Materially the Arterial Pressure in Anesthetized Dogs.

Animal No.	Weight.	Amount of oil necessary to reduce arterial pressure.	Amount of oil per pound of body weight.	Dosage of oil injections.	Frequency of injections.	Total amount of oil injected.	Initial injection of Witte's peptone.
	lbs.	*cc.*	*cc.*	*cc.*	*min.*	*cc.*	
4	19	21	1.1	2–5	1–2	26	No injection.
5	35	29	0.8	2	2–4	37	" "
6	24	23	0.9	2	3–4	23	" "
7	34	35	1.0	5	1–5	55	Injection.
8	27	28	1.0	2	2	28	No injection.
9	30	42	1.4	2	1	44	" "
10	30	25	0.8	2–4	1	38	Injection.
1	18	20	1.1	2	1	28	No injection.
3	42	50	1.2	5	1–3	55	Injection.
11	15	16	1.0	1–2	1	19	"
12	18	18	1.0	2	1–2	18	"
13	30	70	2.3	2–10	1–9	70	No injection.
Average (omitting Dog 13) . .			1.0+				

animal; that is, a 25 pound dog would require about 25 cc. of oil, and a 150 pound man about 150 cc., to induce a lasting fall in pressure, if the same quantitative relations hold for man as for the dog.

It apparently makes only slight difference whether this critical quantity is injected in amounts of 1 or 2 cc. of oil at intervals of 1 or 2 minutes, or in quantities of 5 to 10 cc. at a time. When the larger amounts are injected, with short intervals between the injections, the mechanism of adaptation is put to a greater strain, the critical point may be slightly lowered, the immediate fall is more marked, and the recovery more slow (Fig. 5). But even when relatively enormous quantities of oil are injected at one time and fairly rapidly (for example, in one experiment, 75 cc. of oil were injected very rapidly into a 29 pound dog from a burette connected with the cannula in the femoral vein) the fall in pressure is much more gradual than in the case of peptone or anaphylactic shock (Fig. 6).

Several factors of safety, which in peptone shock appear to be entirely useless, are thoroughly effective in fat embolism in the dog. In the first place, many years ago, Lichtheim[20] showed that three-fourths of the pulmonary circulation may be occluded without affecting the systemic pressure. Tigerstedt[21] and Gerhardt[22] have both confirmed this observation, although Gerhardt, who worked with spontaneously breathing rabbits, questioned Lichtheim's explanation of the phenomenon. More recently, Kuno[23] has shown that the lungs may contain from 8.8 to 19.44 per cent of the amount of blood in the body, depending upon the condition of the circulation.

It is evident, therefore, that much of the vascular space of the lungs can be closed without causing a fall in arterial pressure, a conclusion amply justified by the results here reported. This is due in part to the presence of an excessively large vascular area in the lungs in which there is probably ordinarily much dead space where the circulation is not active. But the occlusion may involve more than this

[20] Lichtheim, L., Die Störungen des Lungenkreislaufes und ihr Einfluss auf den Blutdruck, Breslau, 1876, cited by Tigerstedt, R., *Ergebn. Physiol.*, 1903, ii, pt. 2, 528.

[21] Tigerstedt, R., *Ergebn. Physiol.*, 1903, ii, pt. 2, 528; *Skand. Arch. Physiol.*, 1903, xiv, 259.

[22] Gerhardt, D., *Z. klin. Med.*, 1904, lv, 195.

[23] Kuno, Y., *J. Physiol.*, 1917, li, 154.

excess of vascular area, and the systemic pressure may still be maintained at the normal level by increased work on the part of the right ventricle. Gerhardt[22] observed a rise in pressure in the pulmonary artery under these conditions. Furthermore, the capillaries of the lungs are capable of very great distention, and by dilatation the still unoccluded capillaries can permit the passage of an undiminished or only slightly diminished supply of blood to the left side of the heart.

A more detailed analysis of the curve of arterial pressure obtained during the successive injections of small amounts of olive oil into the veins of a dog shows it to be more complex than the simple curve of peptone shock. In Fig. 2 is shown the effect of injecting a 15 pound dog with 1 or 2 cc. doses of olive oil at intervals of 1 to 2 minutes, until a total of 19 cc. had been injected. It is seen that the first one or two injections produced hardly any perceptible effect. With each succeeding injection the immediate fall in pressure became greater and greater and the return to normal slower and slower. Finally, a point was reached at which, after each injection, the pressure did not again reach its former level. In the case of this particular animal, an injection of peptone had been given, from the effects of which it had not completely recovered when the injections of oil were begun. This accounts for the fact that the arterial pressure continued to rise slowly for some minutes in spite of the injections of oil (see also Fig. 3).

After the critical point had been reached, each succeeding dose of oil brought the pressure lower and lower. If the injections were stopped soon enough, however, the animal could usually be kept alive for some time and the various phases of the condition studied. In all the animals, when the arterial pressure showed a permanent and material depression, however slight at first, the condition gradually became worse, and the animal died in from 1 to 3 hours. If the injections were continued, the animal succumbed very quickly. The anesthetic doubtless played a part in the progressively fatal course.

The interpretation of these results is, perhaps, obvious. It would seem to be a legitimate conclusion that the oil first injected merely filled some of the excess vascular space in the lungs. When this excess was used up, the unoccluded capillaries dilated sufficiently to permit the passage of an undiminished or only slightly diminished

volume of blood. When the capillary area was still further reduced, the right ventricle was still able, by increasing its work, to deliver the necessary amount of blood to the left side of the heart. It is not to be supposed, however, that each of these factors of safety came into action separately and in the order named. They probably all became active early, but in varying degrees. The increasing extent of fall and slower recovery after each succeeding injection was due to the increasing difficulty of readjustment of these various factors to the augmented load placed upon them. A point was ultimately reached at which the fall in pressure induced by each injection was roughly proportional to the amount of reduction in the remaining vascular space in the lungs by that injection. That this was probably true was indicated by the great difference in the amount of fall in pressure induced by the same volume of oil injected before and after the critical quantity had been reached (Figs. 2 and 3). The progressively downward course was probably the result of a break in compensation on the part of the various adaptive factors of safety.

Differences in the Effect of Peptone Shock and Experimental Fat Embolism upon Venous Pressure.

The differences in the effects of peptone shock and experimental fat embolism upon venous pressure are equally striking. In the former, there is usually a slight preliminary rise due to the volume of fluid suddenly injected into the veins with the peptone. This is followed by a precipitate fall. The arterial and venous pressures thus run practically parallel (Fig. 1). While the arterial pressure is gradually rising as the animal recovers, the venous pressure also rises. The onset of dyspnea during peptone shock causes a further fall in venous pressure and a rise in arterial pressure as already described.

In experimental fat embolism, the venous and arterial pressures change in opposite directions. During the successive injections of small amounts of oil there occurs usually a slight temporary rise in venous pressure simultaneously with the slight evanescent fall in arterial pressure. But both quickly return to normal. It is not until the critical quantity of oil has been injected that there is a lasting rise in venous pressure. A relatively small amount of oil in-

jected at this time will cause a rapid rise in venous pressure synchronous with the marked fall in arterial pressure described above (Figs. 2 and 3).

In peptone shock the venous pressure falls because of the sudden stagnation of blood in the liver and organs of the splanchnic area. It does not reach the larger veins which are relatively collapsed. In fat embolism the blood cannot reach the systemic circulation because of the blocking of its passage through the lungs. The blood of the body, therefore, accumulates in the veins. Since all the vessels still retain their normal tone, the venous pressure rises.

It seems obvious that if a sufficient amount of fat has entered the lungs to cause a fall in arterial pressure in any case of surgical shock, there should also be present at the same time an elevation of venous pressure, for in experimental fat embolism in the dog the venous pressure begins to rise only when the arterial pressure begins to fall. There has not been opportunity to make observations upon this point on human cases of shock.

Effects of Dyspnea in Peptone Shock and in Experimental Fat Embolism.

In two papers published in 1916, it was shown that dyspnea will cause a rise in arterial pressure in anaphylactic and peptone shock.[24] Weil,[13] who appears to have seen only the first of these papers in which the idea was not fully developed, has recently stated that "this novel hypothesis is quite unsupported by confirmatory experiments." It seems advisable, therefore, to restate briefly the evidence upon which this claim was made. The phenomenon was first observed in the course of experiments to determine the state of vasomotor irritability in anaphylactic shock in dogs. It was noticed that during the stage of low blood pressure, doses of 1 cc. of a 1:4,000 solution of nicotine sometimes produced a greatly exaggerated reaction. When this occurred the percentile rise[25] varied from 30 per cent to more than 200 per cent, as contrasted with a percentile rise of 10 to 17 per cent before the induction of shock, and with an average percentile rise of less than 10 per cent after its production in those instances in which nicotine did not bring about dyspnea (Table II). It was observed further, that whenever this augmented reaction occurred, the injection of nicotine was invariably followed by more or less dyspnea, and that the rise in pressure was roughly proportional to the severity of the dyspnea.

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[24] Simonds. J. P., *J. Infect. Dis.*, 1916, xix, 746; *Arch. Int. Med.*, 1916, xviii, 848.

[25] Porter, W. T., *Am. J. Physiol.*, 1907–08, xx, 399; 1914, xxxiii, 373.

Dyspnea induced by other means, such as increasing the amount of carbon dioxide in the inspired air, and stimulation of an afferent nerve, has given similar results. But there is no method of causing dyspnea in an anesthetized dog that is entirely free from criticism. Asphyxia is known to cause a rise in blood pressure in the normal anesthetized animal. In the stimulation of an afferent nerve it is not always easy to determine for a given animal the exact strength of current that will produce the maximum of dyspnea with a minimum of pressor or depressor effect.

There is reason to believe that for the purpose of determining the effect of dyspnea upon the low blood pressure associated with peptone shock, that induced by the injections of nicotine may be freer from criticism than that brought about by any other method. It has been shown by Hoskins and Ranson[26] that nicotine causes vasoconstriction chiefly by its action upon the sympathetic ganglia, but partly by its action upon the vasomotor center. Adrenalin, on the other hand, produces a rise in blood pressure as a result of its action upon the nerve endings in the vessel wall.[27] Hence nicotine, as a rule, cannot produce a rise in blood pressure in those conditions in which adrenalin is completely ineffective. In anaphylactic shock and in peptone poisoning, injections of adrenalin are without appreciable effect until the blood pressure has begun to rise. Even when the reaction does return, the percentile rise is reduced, and only gradually approaches the normal for the given animal as the absolute pressure nears its former level. The reaction to nicotine, when unaccompanied by dyspnea, follows an exactly similar course; that is, the actual and percentile rises in blood pressure following nicotine injections (without dyspnea) gradually increase from zero to their pre-shock values as the absolute pressure rises to its normal, as shown in Table II. This is especially evident when the results of injections of nicotine in spontaneously breathing animals are compared with those obtained in dogs similarly treated but with the thorax open and artificial respiration employed. The fact that the actual and percentile rises caused by injections of adrenalin and nicotine, when

[26] Hoskins, R. G., and Ranson, S. W., *J. Pharm. and Exp. Therap.*, 1915, vii, 375.

[27] Dixon, W. E., *J. Physiol.*, 1904, xxx, 97.

TABLE II.

Effects of Injections of Nicotine with and without Dyspnea upon Low Blood Pressure Associated with Anaphylactic and Peptone Shock.*

Animal No.	Before induction of peptone or anaphylactic shock.				After induction of peptone or anaphylactic shock.				
	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Dyspnea.
	mm.	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*mm.*	*per cent*	
14	110	125	15	10	35	35	0	0	None.
					35	120	85	243	Violent.
					35	75	40	114	Marked.
15	105	115	10	10	15	20	5	33	Slight.
					35	90	55	157	Marked.
					75	80	5	7	None.
					75	115	40	53	Moderate.
16	125	145	20	16	30	30	0	0	None.
					50	85	35	70	Moderate.
					80	120	40	50	"
17	140	150	10	7	35	70	35	100	Marked.
					20	70	50	250	Violent.
					80	115	35	44	Moderate.
18	180	200	20	11	35	38	3	9	None.
					30	40	10	33	Slight.
					55	100	45	80	Moderate.
19	100	110	10	10	20	25	5	25	Marked.
					35	50	15	43	"
					45	65	20	44	Moderate.
					55	65	10	18	None.
					60	80	20	33	Slight.
					55	65	10	18	None.
20	160	175	15	9	30	30	0	0	None.
					35	45	10	28	Slight.
					55	65	10	18	None.
					60	120	60	100	Marked.

* Standard dose, 1 cc. of a 1:4,000 solution of nicotine intravenously.

TABLE II—*Concluded.*

Animal No.	Before induction of peptone or anaphylactic shock.				After induction of peptone or anaphylactic shock.				
	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Dyspnea.
	mm.	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*mm.*	*per cent*	
21	100	110	10	10	55	55	0	0	Chest open. Artificial respiration.
					45	47	2	4	
					65	70	5	8	
					52	56	4	8	
					65	70	5	8	
22	120	140	20	17	25	25	0	0	Chest open. Artificial respiration.
					50	52	2	4	
					65	70	5	8	
					85	95	10	12	
					105	120	15	14	

unaccompanied by dyspnea, in anaphylactic and peptone shock run parallel courses, indicates that the direct pressor action of nicotine is inhibited by the same condition that renders adrenalin ineffective. It is for these reasons that in anaphylactic and peptone shock we consider the exaggerated rise in blood pressure induced by nicotine as a more purely mechanical effect than a similar rise accompanying dyspnea brought about by any other method.

We may summarize the evidence that the augmented reaction to nicotine frequently observed in the condition of low blood pressure associated with peptone poisoning is due wholly to the mechanical effect of the dyspnea, as follows: (1) It occurs only in the stage of low blood pressure, and only when the dose of nicotine causes dyspnea. It does not appear when the blood pressure is normal even if dyspnea is produced. (2) It has not been observed in any animal in which the chest has been opened and artificial respiration employed. (3) It is not due to any cumulative effect because of the slowed circulation, for a double dose of the drug will not cause such an augmented reaction before the condition of shock is induced. During the condition of shock a 2 cc. dose is more likely to produce dyspnea and is therefore more frequently followed by a magnified rise in pressure

than is a 1 cc. dose. A double dose without dyspnea does not yield the normal, *i.e.* pre-shock, percentile rise. (4) This augmented reaction occurs at a stage in the condition of shock when adrenalin is without effect, and when, from our knowledge of the pharmacologic action of nicotine,[26] no response to injections of that drug is to be expected. (5) With a large reservoir of stagnating blood in the liver, conditions are favorable for the most effective results from increased respiratory suction and from the force-pump action of the vigorously contracting diaphragm. The distance from the right auricle to the point of entry of the hepatic vein into the inferior vena cava is short. The latter vessel is prevented from collapsing by its attachment to the central tendon of the diaphragm through which it passes. By this pump-like action increased amounts of blood are brought to the underfilled right ventricle and delivered at once through the unimpeded pulmonary circulation to the left ventricle and thence to the systemic circulation, with the resultant rise in pressure. When the blood pressure is normal, dyspnea does not cause this marked rise because there is no large convenient reservoir of blood for respiratory suction to act upon, and because the right side of the heart is already filling properly with each diastole.

During the first ½ minute or so after the blood pressure approaches or reaches its minimum in anaphylactic or peptone shock, dyspnea frequently is not effective in causing a rise in pressure. This is especially well seen by contrasting the results of injections of nicotine in Dogs 17 and 19 (Table II). With a pressure of 20 mm. of mercury immediately before the injection of the second dose of nicotine (11 minutes after injecting 5 cc. of normal horse serum) in Dog 17, a violent dyspnea produced a rise of 250 per cent. With the same initial pressure in Dog 19, between ½ and 1 minute after giving 5 cc. of horse serum, a rise of only 25 per cent was produced in spite of the marked dyspnea which the injection of nicotine caused.

The type of the rise in blood pressure which accompanies dyspnea in peptone shock varies with the severity of the dyspnea. If this is violent, as in Dog 14 (Fig. 4), the rise is rapid; if only moderate, as in Dog 17, the rise is somewhat less steep.[28] The degree of dyspnea does

[28] The tracing from this dog was published in connection with the paper on "Anaphylactic shock in dogs," *J. Infect. Dis.*, 1916, xix, 746.

not affect the percentile rise so much as it affects the rate at which the rise occurs. In either case, there is, in the early stages, a tendency for the pressure to fall again, but usually less rapidly than it rose. As a rule, the pressure does not again return to its former low level, unless the dose of peptone was very large. It then not infrequently falls to a still lower plane. For example, Dog 17, with a blood pressure of only 20 mm. of mercury at the time of the induction of the second period of dyspnea, by nicotine, was apparently *in extremis*. The dyspnea was violent and induced a rise in pressure of 250 per cent, and the animal recovered in a relatively short time. Similar results, although usually less spectacular, have been obtained in many animals.

Dyspnea is, therefore, an important therapeutic agent in low blood pressures of the type present in anaphylactic and peptone shock. By bringing the pressure above the danger zone at frequent intervals by the repeated induction of short periods of dyspnea, the life of the animal can usually be saved. Whether equally beneficial results will follow its use in surgical shock in human patients will depend upon the mechanism of that condition. If the low blood pressure in surgical shock resembles that in peptone poisoning in that it is accompanied by a reservoir of stagnating blood in the liver, it would seem reasonable to expect salutary results from the frequent induction of periods of dyspnea by some method which can be applied with safety in human cases, such as increasing the carbon dioxide content of the inspired air. If, on the other hand, surgical shock has an entirely different mechanism, as, for example, fat embolism, little permanent benefit can be expected from the use of dyspnea. Incidentally it may be remarked that the study of the effects of dyspnea in surgical shock in man may yield information of value in determining the nature and mechanism of that condition.

Since I had observed that dyspnea causes a rise in blood pressure in anaphylactic and peptone shock,[24] Porter's[29] report that dyspnea causes a rise in the low blood pressure accompanying fat embolism led to the present comparative study of these two conditions. My experiments have confirmed Porter's observation, although the re-

[29] Porter, W. T., *Boston Med. and Surg. J.*, 1917, clxxvi, 699.

sults are far less striking than in peptone shock. The character of the curve is different from that obtained in peptone shock. In experimental fat embolism, the rise induced by dyspnea is usually less marked, the ascent is more gradual, and the decline usually slower. The pressure tends to fall progressively lower between the periods of dyspnea, so that the tendency to recover is lacking. All the animals of this series whose blood pressures showed a permanent fall invariably succumbed. In several instances dyspnea appeared to hasten the end by causing acute pulmonary edema. The increased negative intraalveolar pressure as a result of the deeper and more forcible inspirations may have been a factor in the production of this edema as in the case of adrenalin pulmonary edema in rabbits described by Auer and Gates.[30]

Artificial respiration with a bellows has been found to cause a rise in pressure in experimental fat embolism in some dogs. This rise, when it does occur, is very gradual (Figs. 7 and 8).

In view of the marked differences in the state of the circulation in these two conditions, to explain the rise in arterial pressure accompanying or following dyspnea in fat embolism upon the same basis as the similar phenomenon in peptone shock, namely as a result of respiratory suction, as is done by Porter,[29] would hardly seem justified. Conditions are certainly not favorable in fat embolism for the activity of this force. (1) There in no convenient large reservoir of stagnating blood upon which respiratory suction can be exerted. Instead, there is a general venous stasis. The distribution of the blood in the body can be influenced by gravity. Thus, if the foot of the operating board is raised, the venous sinuses of the brain will be found at autopsy greatly distended with blood. If at autopsy the skull is opened before the thorax is disturbed, enormous quantities of blood flow from the opened sinuses, indicating the extreme degree of venous stasis. This does not occur in anaphylactic and peptone shock. (2) The right side of the heart is already overfilled with blood. To add more blood would only increase its burden and augment the tendency, already present, to acute dilatation. (3) A large portion of the vascular bed of the lungs is occluded. Any additional blood brought to the

[30] Auer, J., and Gates, F. L., *J. Exp. Med.*, 1917, xxvi, 201.

heart by respiratory suction or any other force could not be delivered to the left ventricle where it is needed to raise arterial pressure. (4) The venous pressure rises when the arterial pressure falls (Figs. 2 and 3). (5) The vessels still retain their functional integrity as shown by the reaction to adrenalin (Fig. 8).

A more reasonable explanation, but one difficult to verify, is that in experimental fat embolism dyspnea, and perhaps artificial respiration with a bellows, in some way facilitates the passage of blood through the lungs. Cloetta[31] has called attention to the effect of inflation of the alveoli upon the caliber of the interalveolar capillaries. When the lung is collapsed, the capillaries are reduced in diameter. With moderate inflation there is radial traction upon these vessels and their lumina are increased in size. With marked inflation, the capillaries are narrowed, both by compression and by linear extension. In dyspnea this cycle is repeated in rapid succession, so that a milking action upon the capillaries is produced which would tend to dislodge mechanically the occluding droplets of oil from these vessels. But the question of the mechanism by which dyspnea and artificial respiration cause a rise in blood pressure in experimental fat embolism must remain for the present without a satisfactory answer.

We may summarize the effects of dyspnea upon the low blood pressures in peptone and anaphylactic shock and in experimental fat embolism as follows: In the former, the rise in pressure is relatively sharp; there is a tendency to decline, but the pressure does not usually reach its former low level unless the dose of peptone was exceptionally large; and the animal generally recovers if the periods of dyspnea are repeated with sufficient frequency to prevent serious damage to the vital centers of the brain by the anemia. In experimental fat embolism, the rise in pressure accompanying dyspnea and the subsequent decline are usually more gradual than in peptone shock; the tendency is for the pressure to sink progressively lower, after each paroxysm of dyspnea; and no permanent benefit has been observed from the employment of dyspnea after the arterial pressure has once been materially and permanently reduced. If permanent

[31] Cloetta, M., *Arch. exp. Path. u. Pharm.*, 1912, lxx, 407.

improvement in surgical shock is found to follow repeated periods of dyspnea, this would appear to be indirect evidence that this condition is not due to fat embolism.

Further Differences between the Low Blood Pressure Associated with Peptone Shock and Experimental Fat Embolism.

In peptone and anaphylactic shock the respiration is usually not affected except for the temporary dyspnea that occasionally occurs during the fall in pressure. In experimental fat embolism, on the other hand, two very unlike changes in respiration have been observed in different animals. Sometimes both conditions have developed in the same animal at different stages of the experiment. In a number of dogs a violent dyspnea occurred, lasted for several minutes, and usually resulted fatally. This was almost always accompanied by edema of the lungs. The condition closely simulated the clinical picture of fat embolism sometimes seen after fractures of long bones. In several instances the difficulty of respiration became so extreme that it was necessary to disconnect the tracheal cannula from the ether bottle. With each violent expiration a shower of frothy fluid was blown from the cannula. The degree of edema appeared to bear a direct relation to the severity of the dyspnea.

In other animals a condition of apnea not infrequently developed unexpectedly without any change in the amount of anesthetic being given. This may occur before the critical quantity of oil has been reached in the injections, and therefore before the arterial pressure has begun to fall. The onset of apnea may be sudden, but usually it is somewhat gradual. The respirations become more shallow and less frequent until they stop entirely, as in fatal ether poisoning. If the anesthetic is removed promptly and moderate artificial respiration instituted at once, the animal can usually be revived, and after a varying period of time will begin to breathe spontaneously again. If the artificial respiration is not started without delay, the blood pressure quickly falls and the heart stops beating within a few minutes. A delay of $\frac{1}{2}$ minute has appeared, in some instances, to result in the death of an animal that might have been revived. This apnea has not been observed in peptone shock.

The similarity of this series of events to ether poisoning, and the recovery of the animal upon the removal of the anesthetic and the institution of artificial respiration, has led to the tentative explanation that the toxicity of ether may be enhanced in experimental fat embolism. The recovery of the dog under the conditions noted would seem to exclude the possibility of any organic damage to the respiratory center by the lodgment therein of an embolus of oil. Careful postmortem examination, gross and microscopic, of the region of the floor of the fourth ventricle has not revealed evidence of such a lesion.

Janeway and Jackson[3] reduced arterial pressure by compressing the inferior vena cava. Release of the compression after 2 hours was followed by a prompt rise in arterial pressure, which slowly fell again, and the animal died in about 12 hours. They consider that the dilatation of the peripheral venules and capillaries as a result of the increased venous pressure caused such a loss of tone that even after normal pressure was restored they became overfilled with blood. Too little blood was thus permitted to reach the heart to keep the arterial pressure above the danger zone of 40 to 50 mm. of mercury, and the animal died.

The animals used in this series of experiments were observed for varying periods of time after the injection of oil. In most instances the experiments did not last longer than 5 hours. Hence from the observations of Janeway and Jackson, it might be objected that an amount of fat in the lungs less than the critical quantity may cause a fall in pressure more than 5 hours after the injection. That there is a rise in venous pressure in experimental fat embolism is known from the work of Warthin,[4] and of Bissell,[5] and from the results here reported. But as far as these experiments go, it would seem that a rise in venous pressure of a lasting character or of a degree greater than the fluctuations observed in dogs without any further treatment than the administration of an anesthetic, does not begin until the critical quantity of oil has been injected and the arterial pressure has begun to fall. Even then the venous pressure (external jugular-superior vena cava) may be lowered by dyspnea. The conditions present in experimental fat embolism do not, therefore, appear to be analogous to those in the experiments of Janeway and Jackson until a relatively large amount of oil has been administered. After

this point has been reached, that is after the venous pressure has begun to rise, progress to a fatal termination is usually rapid. Hence there is no reason to consider this possible objection valid.

SUMMARY.

1. In peptone shock there is a marked, precipitate fall in arterial pressure. At the same time there is a fall in venous pressure.

2. In experimental fat embolism, (*a*) the fall in blood pressure is always gradual; (*b*) approximately 1 cc. of oil for each pound of body weight must be injected before a lasting fall in arterial pressure is produced; (*c*) it makes only a slight difference whether this amount is injected in small doses at a time or in relatively large quantities; and (*d*) when the arterial pressure falls, but not till then, the venous pressure rises.

3. In peptone shock, dyspnea, by its suction and force-pump action upon the reservoir of stagnating blood in the liver, brings more blood to the heart and causes a rise in arterial pressure. By repeatedly inducing short periods of dyspnea at frequent intervals, permanently beneficial results are obtained and the life of the animal can be saved.

4. In experimental fat embolism, dyspnea will cause a rise in blood pressure. But permanently beneficial results have not been obtained by this method. If dyspnea is found to bring permanent improvement in surgical shock, it is indirect evidence that this condition is not due to fat embolism. Respiratory suction is probably not responsible for the rise in blood pressure in experimental fat embolism. It seems more likely that the dyspnea in some way facilitates the passage of blood through the embarrassed pulmonary circulation. Artificial respiration with a bellows will also frequently cause a rise in blood pressure in experimental fat embolism.

5. In peptone shock the respiration is usually not affected, although there is some evidence that the respiratory center may be in a state of increased irritability. In experimental fat embolism, in some animals a violent dyspnea develops spontaneously. This is usually accompanied by edema of the lungs. In other instances, an apnea occurs, even before the blood pressure has begun to decline.

EXPLANATION OF PLATES.

PLATE 17.

FIG. 1. The fall in arterial and venous pressures associated with peptone shock. Dog 3; weight 42 pounds. The writing point of the venous pressure manometer was recording $\frac{3}{4}$ inch in advance of that of the arterial manometer.

FIG. 2. The effect on arterial and venous pressures of injecting small doses of oil at frequent intervals. This animal had been previously subjected to peptone shock, which accounts for the gradual rise in arterial pressure in the first section of the tracing (*a*), in spite of the injections of oil. Between the first and second (*b*) sections of the tracing, 4 minutes elapsed and 6 cc. of oil were injected. After the arterial pressure had begun to fall, the respiration became slow and four inspirations with the bellows were used as shown in the second section of the tracing. Dog 11; weight 15 pounds.

PLATE 18.

FIG. 3. The effect on arterial and venous pressures of the injection of small doses of oil at frequent intervals. The same animal as in Fig. 1. Between the first (*a*) and second (*b*) sections of the tracing 20 minutes elapsed. During this time a clot formed in the venous cannula. In removing this clot some fluid was lost from the system of transmission. This probably accounts for the lower level of the venous pressure tracing in this section of the figure. In the second section the arrows indicate corresponding positions of the writing points of the two manometers.

PLATE 19.

FIG. 4. Dog 14. The effect of nicotine on arterial pressure in peptone shock with and without the production of dyspnea. *a* and *b*, injections of adrenalin and nicotine, respectively, before the induction of shock; *c*, peptone injected; *d*, adrenalin; *e*, nicotine without dyspnea; *f*, nicotine with marked dyspnea. Dosage 1 cc. of 1:50,000 adrenalin, and 1 cc. of 1:4,000 nicotine.

FIG. 5. The effect of rapidly injecting large single doses of oil at frequent intervals, the total amount being less than the critical quantity. Moderately rapid fall in pressure with gradual rise to previous level. Dog 23; weight 30 pounds. The time covered by the tracing, including the period during which the drum was stationary, was 18 minutes.

PLATE 20.

FIG. 6. Gradual fall in arterial pressure after rapid injections of large amounts of olive oil (75 cc.). The effect on respiration is also shown. Female dog; weight 29 pounds. Time covered by entire tracing, 7 minutes.

FIG. 7. The effect of artificial respiration with a bellows upon low blood pressure (arterial) in experimental fat embolism. The animal was breathing spontaneously when the artificial respiration was instituted. Time covered by tracing, 4 minutes.

PLATE 21.

FIG. 8. The effect of artificial respiration with a bellows upon low arterial pressure in experimental fat embolism. The respiration became very slow and finally stopped. Rapid fall in pressure, followed by gradual rise during artificial respiration. Between the first (*a*) and second (*b*) sections of the tracing $3\frac{1}{2}$ minutes elapsed. During this time the pressure continued to rise gradually. Respiration became spontaneous while the drum was stopped. The rise in pressure indicated by the perpendicular line occurred very quickly upon the suspension of artificial respiration before the drum could be started. This tracing also shows the reaction to adrenalin in the low blood pressure due to experimental fat embolism in contrast to the absence of this reaction in peptone shock.

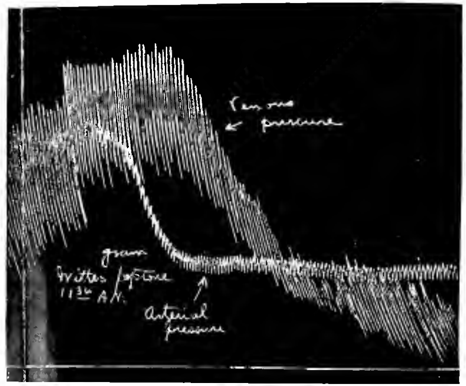
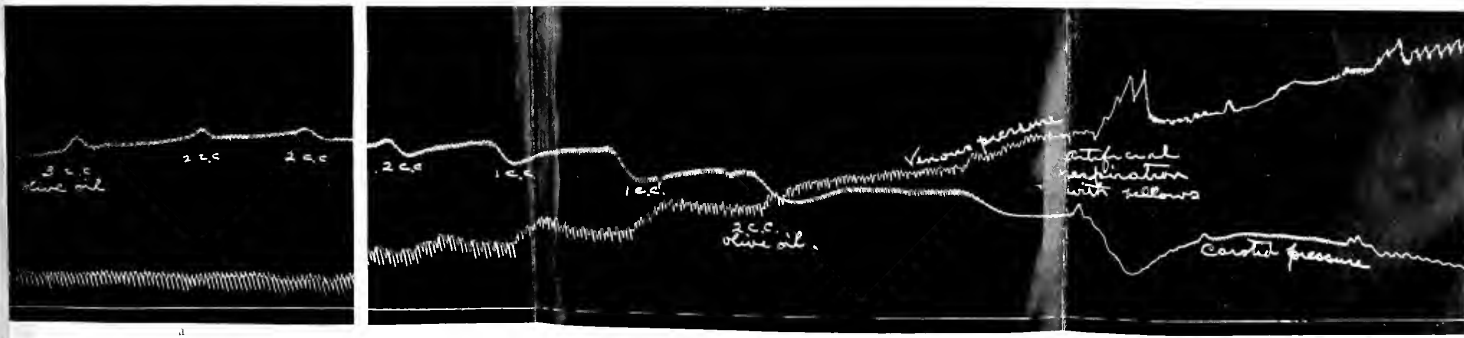

Venous pressure
Witte's peptone
Arterial pressure

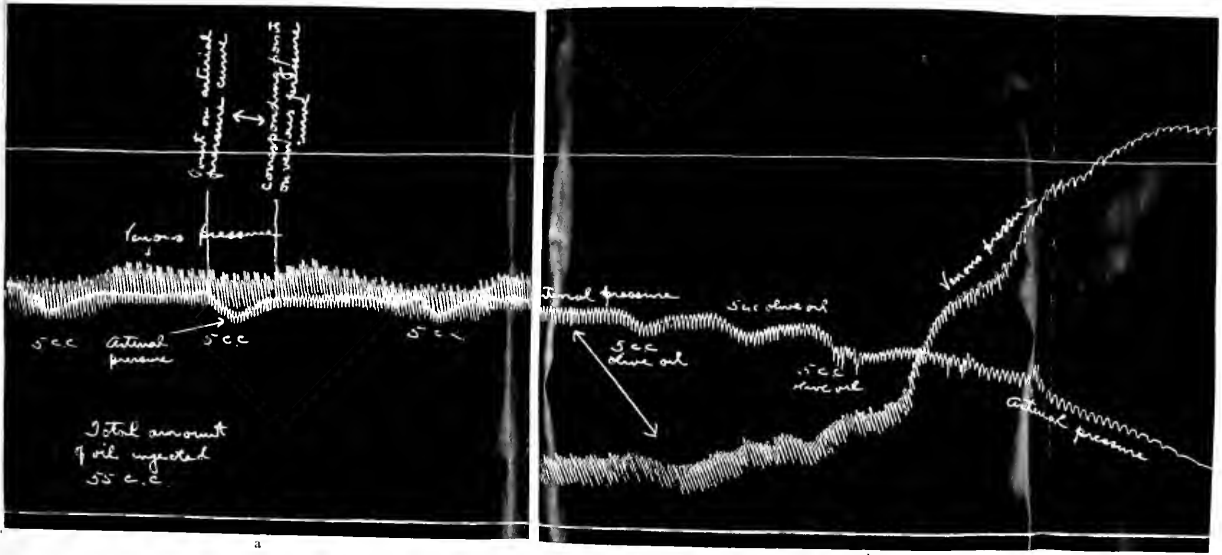
Fig. 1.


3 c.c. olive oil
2 c.c
2 c.c
2 c.c
1 c.c
1 c.c.
2 c.c. olive oil
Venous pressure
artificial respiration with bellows
Carotid pressure

a

b

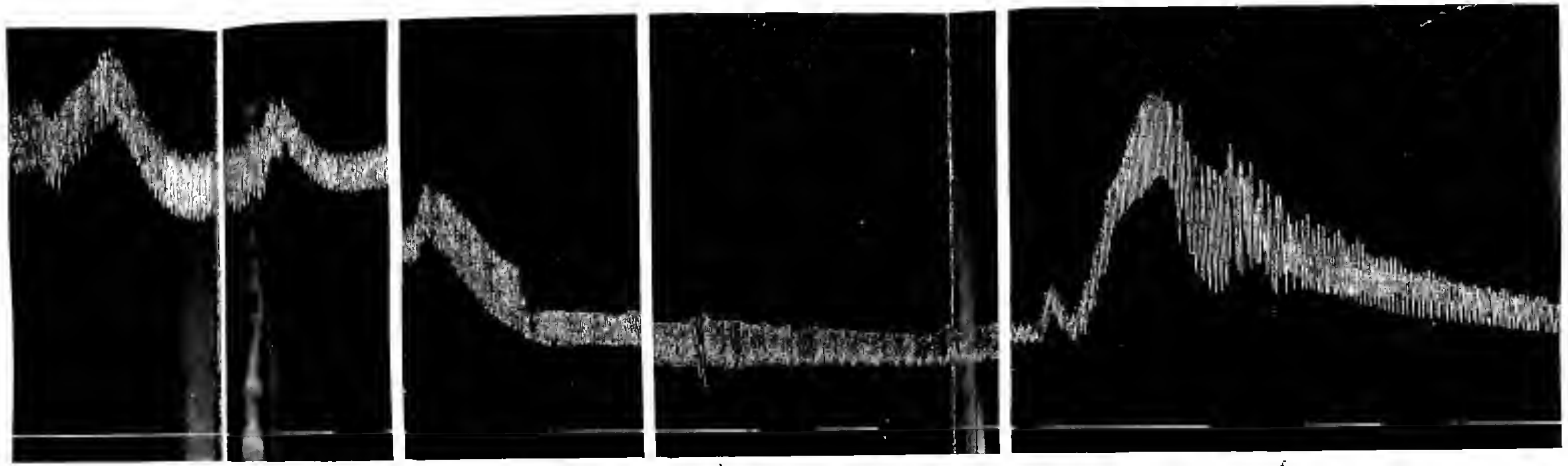
Fig. 2


Point on arterial pressure curve
Corresponding point on venous pressure curve
Venous pressure
5 c.c
Arterial pressure
5 c.c
5 c.c
Total amount of oil injected 55 c.c
Venous pressure
5 c.c olive oil
5 c.c olive oil
5 c.c olive oil
Venous pressure
Arterial pressure

a

b

FIG. 3.



a b c d e f

FIG. 4.

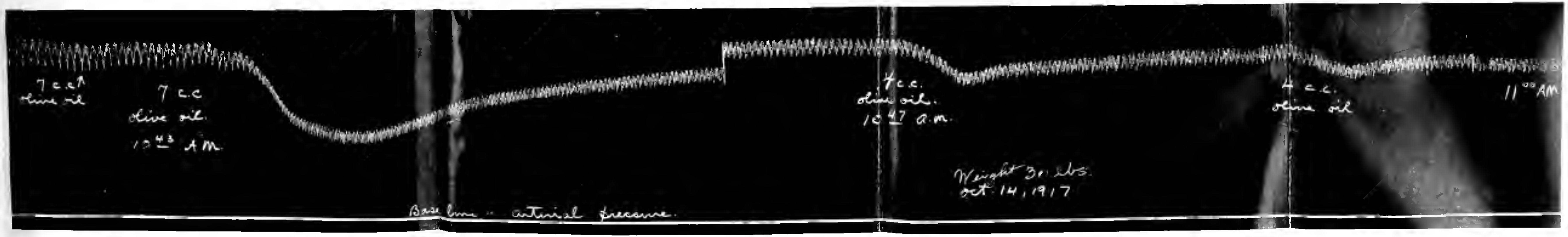


FIG. 5.

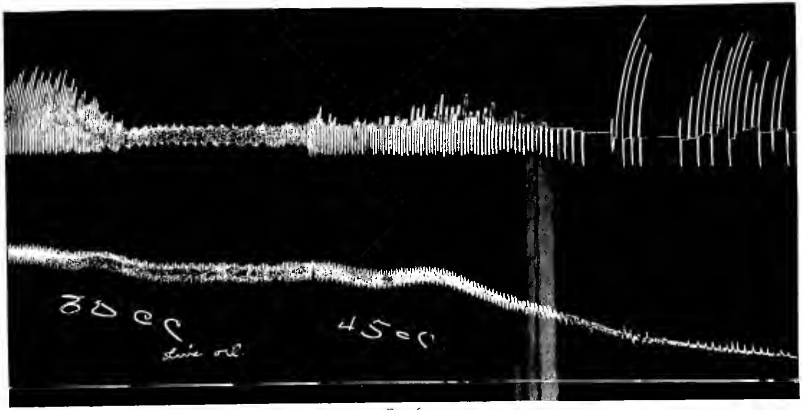


FIG. 6.

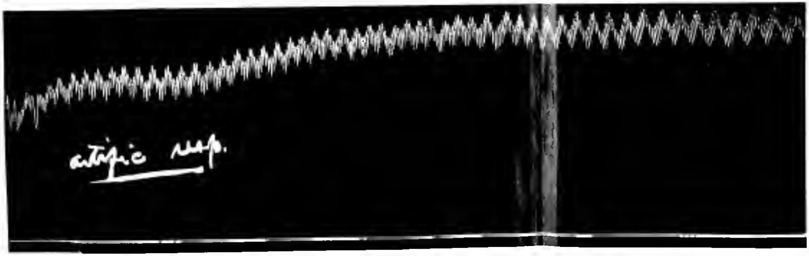
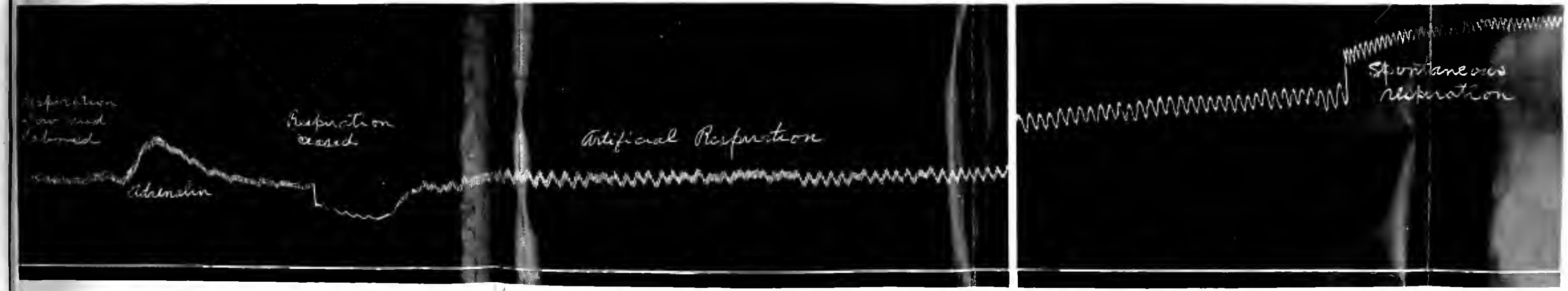


FIG. 7.

(Simonds: Low blood pressure.)



a b

Fig. 8.

(Simo... Low blood ...

AUTOHEMAGGLUTINATION EXPERIMENTALLY INDUCED BY THE REPEATED WITHDRAWAL OF BLOOD.

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Plate 22.

(Received for publication, March 11, 1918.)

There is much evidence in the literature to show that moderate losses of blood act to increase the titer of antibodies developed in response to an injected antigen.[1] Little attention, though, has been given to whether the production of antibodies normal to the organism is likewise stimulated. The point has considerable clinical interest, especially in connection with the remarkable resistance to infection manifested by many patients with severe anemia. In the work now reported one phase of the problem has been taken up; namely, the influence of repeated bleedings on the normal isohemagglutinins.

Experiment 1.—Interagglutination tests were carried out with the cells and sera of twelve normal rabbits. The cells had been twice washed and made to 5 per cent suspensions with salt solution. The sera were obtained from the clot at room temperature (22°C). Mixtures of each serum with each cell suspension in equal parts were kept at 22°C. for 15 minutes and examined with the microscope. Agglutination was found in a moderate proportion of the 144 mixtures. Autoagglutination was not noted.

On the basis of the findings, the animals were separated into two groups, each possessing about the same proportion of material for isoagglutination; that is to say, susceptible cells and agglutinating sera. One group of six rabbits was set aside as controls, and the remaining six rabbits were bled 10 cc. from the heart every 3 to 6 days during a period of 2 months. All were kept under the same conditions. From time to time the interagglutination tests were repeated, sometimes by the method just described, sometimes by mixing the whole citrated bloods in the proportions of 9 to 1 and 1 to 9, according to the method of Rous and Turner.[2]

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[1] See Lippmann, *Z. exp. Path. u. Therap.*, 1914, xvi, 124, for large bibliography.

[2] Rous, P., and Turner, J. R., *J. Am. Med. Assn.*, 1915, lxiv, 1980.

Experiment 2.—Fourteen rabbits were used in two groups arranged on the same basis as in Experiment 1, but with only six individuals in the control group. The other eight animals were bled as already described during a period of 26 days. Interagglutination tests were carried out between the control animals and between these and the bled animals, but not between the individuals of this latter sort. Citrated bloods were used in the tests, which were repeated at intervals of a week or more. Tests with sera and washed cells were also made when this seemed advisable. In whole citrated bloods autoagglutination is easily seen. None was discoverable prior to the bleedings.

No Induced Isoagglutinins.

A number of the rabbits possessed isoagglutinins to start with. Some were bled, and some kept as controls. The bleedings had no demonstrable effect to alter the isoantibodies or to cause an appearance of new ones. It is true that weak isoagglutinins sometimes developed in individuals possessing none to begin with, but they were found to practically an equal degree in the controls and were probably in the nature of intercurrent serum changes such as Ottenberg and Thalhimer[3] have reported.

Clumping in the Shed Blood.

In five out of fourteen of the bled rabbits there developed a tendency of the red cells to clump together into masses in the shed blood. The clumping was never general, bringing together all the cells, as in the case of rabbits repeatedly transfused,[4] but the cell masses lay scattered here and there amid free cells. The phenomenon was not found in any of the twelve controls, nor has it since been observed in a large series of other normals. In instances of anemia, on the other hand, resulting from malnutrition, it has sometimes been met with.

The clumping phenomenon was definitely associated with the anemia following in some instances on the bleedings. Many medium sized rabbits withstand excellently the loss of 10 cc. of blood every 3 to 5 days during a long period. Their hemoglobin percentage and the appearance of the corpuscles remain practically unaffected. The clumping was never noted in these animals. In other rabbits the

[3] Ottenberg, R., and Thalhimer, W., *J. Med. Research*, 1915–16, xxxiii, 213.

[4] Rous, P., and Robertson, O. H., *J. Exp. Med.*, 1918, xxvii, 509.

bleedings rapidly brought about a moderate anemia with pale corpuscles, microcytes, and poikilocytes in circulation. In such instances the clumping occurred, though it was by no means a regular accompaniment of the condition. The number of bleedings and total loss of blood prior to appearance of the clumping were sometimes surprisingly small. Clumping was well marked in a 1,500 gm. rabbit 3 days after the last of two bleedings of 10 cc. each with an interval of 3 days between. In another animal of 1,350 gm., also bled twice, but at an interval of 7 days, the phenomenon was visible 3 days after the last bleeding. In both cases the blood loss was very poorly borne.

The clumping was plainly apparent in whole citrated bloods[5] allowed to stand for 15 minutes or more at room temperature and examined microscopically after dilution with salt solution. When at all marked it could be seen in thick slide preparations of the blood, as such, providing the cells were not numerous enough to interfere with the observations (Fig. 1). Under these circumstances it appeared within about a minute after the blood was shed and before any clotting had occurred. Each clump consisted of from 3 or 4 to 40 or 50 corpuscles massed helter-skelter.

Cause of the Clumping.

The question whether the phenomenon had its cause in a change in the cells, or plasma, or in both, was largely answered by the routine tests of Experiments 1 and 2. These demonstrated that the cells of the bled rabbits had undergone no alteration as regards agglutinability or inagglutinability by normal sera of known behavior. Furthermore, the clumping, like that due to the autoagglutinin present in normal plasma[6] and the principle present in the plasma of transfused rabbits,[4] did not occur at body heat. The cells remained free in citrated blood at 38°C., and the massing together which was visible at room temperature disappeared when the specimen was warmed. Corpuscles separated from the citrated plasma while warm, then washed in warm salt solution and brought together in it at room temperature,

[5] 10 parts of blood to 1 part of a watery 10 per cent solution of sodium citrate.

[6] Landsteiner, K., *Münch. med. Woch.*, 1903, i, 1812.

failed entirely to clump. But when a little of the thick cell suspension was dropped into the original citrated plasma, the cells massed together at once. All these facts showed that the clumping was due to an element in the serum and that this element has much in common with the normal and induced autoagglutinins.

Distinguishing Traits of the Agglutinin.

The agglutinin of the bled rabbits was able to cause clumping in the whole blood as such, or in the whole citrated blood, at room temperature (22°C.), whereas the normal agglutinin is effective at 22° only when a large amount of serum is allowed to act on a few cells.[5] The agglutinating principle of the bled rabbits, obtained in the free state, as in serum separated from the cells by defibrination and centrifugation at 38°C., was so strong in all cases as to bring about clumping at 22°C. in mixtures of the serum with an equal part of a 5 per cent suspension of the animal's own washed cells, and in some instances when there was a further dilution with one part of salt solution. The serum of five normal rabbits similarly separated and tested yielded not the least trace of agglutination. These results with a constant amount of antigen (the 5 per cent cell suspension) rule out the possibility that clumping in the anemic rabbits was due merely to the action of the normal autoagglutinin on an antigen (the red corpuscles) diminished in quantity by the bleedings.

Attempts to obtain the agglutinating factor in salt solution led to a singular finding. Normal autoagglutinins become much more effective as cooling proceeds from room temperature to 0°C. and are best demonstrated in the cold. The agglutinin of the bled rabbits, on the other hand, while effective at room temperature, may be relatively little enhanced in activity by further cooling, and at 0°C. may be surpassed in its action by the normal antibody.

Experiment 3.—A few cubic centimeters of blood were obtained from each of two normal rabbits and two which had been repeatedly bled and were the possessors of an agglutinin which caused clumping at room temperature. The sera were separated from the cells by defibrination in the warm, and centrifugation in a water jacket at 38°C. Those of the bled animals caused clumping at room temperature when mixed with an equal volume of a 5 per cent suspension of the corresponding cells twice washed in the warm. In similar mixtures of normal

sera no clumping occurred. Now 1.2 cc. of each serum was mixed with 0.1 cc. of a 50 per cent suspension of the corresponding cells, and the tubes were plunged in melting ice. The results are given in Table I.

It will be observed that the agglutinin of the normal rabbit No. 2, while practically ineffective at room temperature (22°C.), caused a more complete clumping at 0°C. than did the agglutinins of the bled rabbits, which were so active at 22°C. A second experiment along these lines gave similar results. The data do not enable one to say whether two distinct sets of antibodies are here concerned, but they

TABLE I.

Rabbit.	Room temperature (22°C.). Microscopic observations after 15 min.				0°C. Macroscopic observations.	
					1.2 cc. serum + 0.1 cc. 50 per cent red cells.	
	Whole blood.	3 parts serum + 1 part 5 per cent red cells.	1 part serum + 1 part 5 per cent red cells.	1 part serum + 1 part salt solution + 1 part 5 per cent red cells.	After 8 min.	After 66 min.
Normal.						
1	0	0	0		+	Sedimentation incomplete; sediment finely granular.
2	0	Tr.	0		++++	Complete sedimentation into a single, solid mass.
Bled.						
3	+−	+	Tr.		+++	Sedimented completely into fairly large masses.
4	+		+	Tr.	++++	Complete sedimentation into a few large masses.

show clearly that the effects of an autohemagglutinin at one temperature cannot safely be taken as the indicator of the effects at another. The study of such antibodies assumes in consequence great complexity.

Agglutination and Rouleau Formation Are Not Connected.

The observations of several authors have led them to conclude that rouleau formation is intimately connected in its cause with agglutination. Our findings in transfused rabbits[4] would seem also to point to this, since the appearance of new agglutinins in the blood is

always preceded by exaggerated rouleau formation. But the present results with rabbits repeatedly bled prove that the association is not obligatory. Here a partial or complete loss of the tendency to rouleau formation was regularly noted to accompany the development of the autoagglutinin (Fig. 1).

SUMMARY.

The repeated withdrawal of moderate quantities of blood does not lead to a development of new isoagglutinins in rabbits, or to noteworthy changes in normal ones already present. On the other hand, clumping of the animals' own cells in specimens of the whole blood is a frequent result. It is found in animals rendered anemic by the bleedings, not in those that rapidly repair their losses and remain in good condition. A similar clumping is sometimes to be seen in the blood of rabbits rendered anemic by malnutrition.

The clumping is due to a true autoagglutinin, which differs from the normal autoagglutinin in its far greater strength, as also, at least in some instances, by a peculiar variation in its activity with changes of temperature.

In the rabbits which developed isoagglutinins after bleeding, the tendency of the cells to form rouleaux was far less than the normal. It follows that rouleau formation is not essentially connected with autoagglutination, as has been assumed in the past.

In the light of the present findings a systematic search for autohemagglutinins in the blood of anemic patients would seem of interest. They have been noted in sick human beings (Ascoli, Klein), but not in recent years. The reason for this may well be that present day blood examinations are not of a sort to bring about their discovery. Thick films of fresh blood are seldom used for clinical purposes, and it is in thick preparations that clumping is most readily observed.

EXPLANATION OF PLATE 22.

Fig. 1. Autoagglutination in the blood of a rabbit rendered anemic by bleeding. Fresh slide-and-cover-glass preparation.

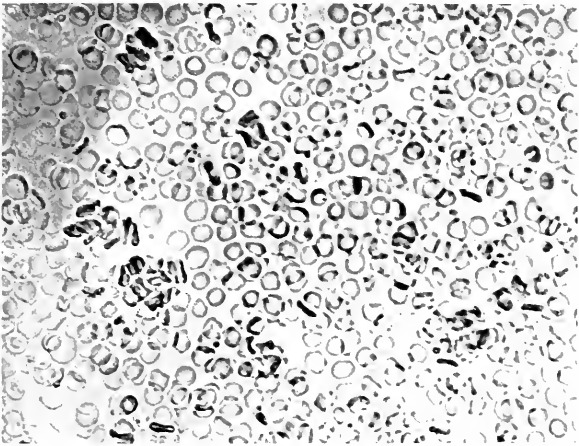

Fig. 1.

(Robertson and Rous: Autohemagglutination.)

EXPÉRIMENTS OUTLINING THE LIMITATIONS OF OPERATIONS ON THE ABDOMINAL AORTA.

By CHARLES GOODMAN, M.D.

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PLATES 23 AND 24.

(Received for publication, January 28, 1918.)

From the results of the five experiments presented in this report, there seems to be no difficulty in correcting injuries of the abdominal aorta in dogs, with subsequent perfect restoration of the continuity of the vessel. The complete occlusion of the aorta for a period of 30 minutes is not necessarily followed by serious consequences.[1] For lateral defects in the aorta, or for injuries not involving the entire circumference, a rectangular clamp may be applied for a prolonged period in order that the circulation should not be completely cut off. An operative field free from blood can thus be obtained while the circulation is maintained through the remaining lumen of the aorta.

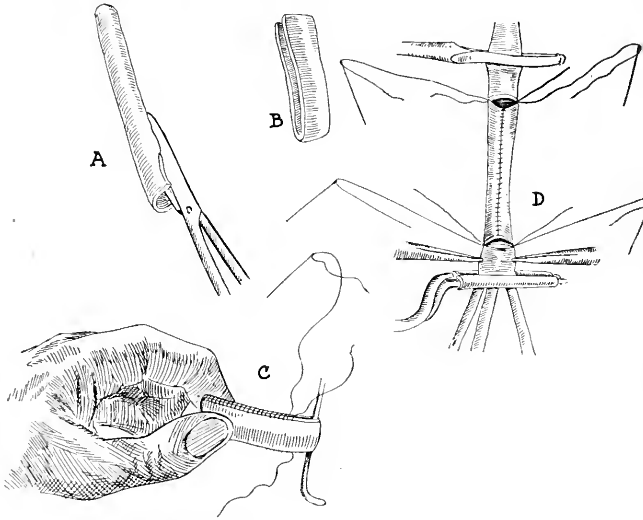
In the instances in which a portion of the aorta must be resected, an arterial segment taken from another animal can be safely utilized as a transplant. Smaller vessels can be adapted to the caliber of the aorta by the following procedure which was devised by Jeger and Helmuth Josef[2] (Text-fig. 1). The artery is split longitudinally (*A*), the ribbon thus formed is then folded double (*B*), and both edges are closed with a continuous suture of very fine silk (*C*). In this way, a tube, double the original size of the vessel is made, which is then transplanted end to end between the resected ends of the aorta (*D*).

Further experiments were undertaken to determine the practicability of reestablishing the continuity of the severed aorta by the cir-

[1] All operations were performed under complete ether anesthesia.

[2] Jeger, E., Die Chirurgie der Blutgefässe und des Herzens, Berlin, 1913.

cular suture. The results obtained seem to indicate that while the operation is possible, the difficulty of approximating the severed ends during the suture is greatly increased, on account of the retraction of the aortic tube. The approximation entails such injury that the probability of thrombosis is much increased. Therefore, when the aorta is completely severed, the introduction of a transplanted seg-



TEXT-FIG. 1. Method for transplanting an arterial segment to the aorta. *A*, first stage of the operation, *B*, second stage, *C*, third stage, and *D*, fourth stage.

ment would be indicated and the procedure would be the same as when a portion of the aorta had been removed.

Danis,[3] in an exhaustive study, showed that the ligature of the abdominal aorta in human beings has hitherto always resulted fatally. Braun[4] has reported a successful suture of the abdominal aorta on a 6½ year old girl with a ganglioneuroma about 4 pounds in weight.

[3] Danis, R., Anastomoses et ligatures vasculaires, Brussels, 1912.

[4] Braun, cited by Jeger,[2] p. 236.

EXPERIMENTS.

Suture of a Longitudinal Incision in the Aorta.

Dog 1.—March 29, 1916. The aorta was exposed below the renal vessels by a median laparotomy. An incision 2 cm. in length was made through all the coats of the right anterolateral wall of the aorta. A rectangular clamp was applied to the aorta in order to shut off the blood from the operative field without completely occluding the aortic circulation. The incision was then closed with a continuous suture of silk. The parietal peritoneum was sutured over the aorta, and the laparotomy wound was closed by suture in layers.

Autopsy.—The dog was killed June 5, with chloroform. There were no evidences of infection. The abdominal wound healed by primary intention. The peritoneum was free from adhesions. The peritoneum covering the aorta was perfectly smooth, and showed no signs of a recent operation.

Gross Examination.—A cross-section of the suture line shows that the vessel is not surrounded by adventitious tissue except at the site of the suture. At this point the vessel wall is but slightly thickened. The intima is relatively smooth except along the suture line. Here there are slight, flat elevations of coagulated blood. These coagulations are old; their surfaces are smooth and they project into the lumen but slightly. New accretions of coagulated blood are apparently taking place at the site of the old blood coagulations described above.

Microscopic Examination.—Section of the vessel wall shows that the structure is intact and the wall preserved. The intima is smooth at the point of this section and shows no plaques of fibrin.

Suture of a Lateral Defect in the Aorta.

Dog 2.—Small female; bulldog type. April 5, 1916. The aorta was exposed through a median laparotomy wound. An elliptical segment of the aorta was excised, and the resulting defect was closed by a continuous longitudinal suture. Although the lumen of the aorta was diminished about one-third of its diameter, the general health of the animal did not seem to be impaired.

Autopsy.—The dog was killed with chloroform April 12. The specimen showed no evidence of thrombosis.

Gross Examination.—At the point of suture the vessel is surrounded by adventitious tissue about 4 cm. thick. The lumen which is present is but slightly if at all diminished in size. The intima is relatively smooth except just at the suture line where it is finely roughened by what appear to be minute plaques of fibrin.

Microscopic Examination.—The section immediately adjacent to the site of suture shows a considerable infiltration of the adventitious tissue by round cells. The structure of the vessel wall is well preserved. At points there is the suggestion of fibrin formation on the intima. In the lumen of a large sized branch of

the aorta at this point is an extensive thrombus. In the many small vessels in the tissue immediately adjacent, the blood circulation has apparently been free.

Homotransplantation of Carotid Tube to Severed Aorta.

Dog 3, Donor.—April 18, 1916. Both carotids were removed. The carotids were slit longitudinally, and the free lateral borders sutured together so as to form an arterial tube twice the caliber of a single carotid. The dog was killed under ether at the operation.

Dog 4, Host.—Small female fox-terrier. A segment of carotid tube 3 cm. long was transplanted by a biterminal suture between the severed ends of the abdominal aorta. The transplantation was made somewhat difficult by inability to control completely the hemorrhage from the aorta during the suture. The immediate circulation, when the operation was completed, was satisfactory. April 20. Animal died.

Autopsy.—No evidences of leakage. Lumen patent. Sutures plainly visible. The specimen showed evidences of lateral thrombosis.

Gross Examination.—The vessel wall is intact but slightly thinner than the normal vessel adjacent. The longitudinal suture lines which have reconstructed the segments of the carotid into the single tube for transplant are plainly visible, though apparently covered by tissue. The intimal surface of the transplant is but slightly roughened by plaques of fibrin. The transverse suture lines are slightly roughened by minute coagula.

Microscopic Examination.—Section across the transverse suture line shows that the intimal surface is covered here and there by a thin layer of coagulated blood. The transplanted segment is somewhat degenerated and infiltrated by round cells. Section through the center of the transplanted segment shows a thin layer of coagulated blood on the intima, and the vessel wall has partially degenerated though it is physically intact (Fig. 1).

Homotransplantation of a Segment of Aorta to the Aorta.

Dog 5.—Medium sized male mongrel. May 17, 1916. The aorta was exposed by a median laparotomy and liberated for a distance of about 5 cm. Two elastic clamps were then applied and the aorta was severed. A segment about 3 cm. long had been removed about an hour before from a small mongrel dog that had been killed by chloroform. This segment was now interposed between the severed ends of the aorta and united by a biterminal suture. After the circular suture was completed, there was no leakage.

Autopsy.—The dog was killed with chloroform June 5. With the exception of the adhesion of the omentum to one point in the line of suture there were no traces of peritoneal irritation. The peritoneum covering the aorta was absolutely smooth. There was some thickening about the transplanted segment. No thrombosis.

Gross Examination.—The aorta is surrounded by a mass of adventitious tissue measuring about 1 cm. in thickness. At the site of the suture a distinct wall of the vessel is apparent, differentiated from the surrounding tissue. The lumen which is present is somewhat diminished in size at the site of repair. There are no blood coagulations in the lumen. The intima appears relatively smooth.

Microscopic Examination.—There is extensive round cell infiltration of the adventitia in and about the sutures. External to the vessel wall are areas of hemorrhage—the result of operation. The vessel wall at this point is intact and whatever intima is shown in the section is free from blood coagulations.

Transplantation of Fascia to a Lateral Defect in the Aorta.

Dog 6.—Medium sized fox-terrier. May 24, 1916. By a median laparotomy the aorta was exposed and liberated for a distance of about 5 cm. A rectangular clamp was applied, and a section $\frac{1}{2}$ cm. square was removed from the anterior wall. This defect was covered by transplanting a piece of aponeurosis taken from the abdominal wall.

Autopsy.—The dog was killed on June 9 with chloroform. The peritoneal cavity was free from adhesions except for a fibrous deposit about the parietal peritoneum corresponding to the field of operation. There were no evidences of leakage from the aorta, and there was no obstruction or thrombosis. The area of intimal surface corresponding to the transplant was perfectly smooth.

Gross Examination.—The specimen consists of a segment of aorta surrounded at the site of fascial transplant by a mass of adventitious tissue. Cross-section shows that the arterial wall at the transplant is deficient and is replaced by scar tissue. The intimal surface over the site of the transplant is covered with a thin layer of fibrin. This surface is relatively smooth. The vessel lumen is in no way diminished at this point. Adjacent to the patch projecting into the lumen is a rounded mass of coagulum about 1 mm. long, apparently a suture end free in the blood stream covered by this blood clot.

Microscopic Examination.—Between the ends of the sutured artery there is a mass of loosely built connective tissue. The surface is smooth where this granulation tissue is exposed to the blood stream. An intact cellular layer of adapted connective tissue cells forms this smooth surface. There is a small island of fibrin attached at one point. In the depths of the artery wall are sections of several sutures. Around the sutures are cellular aggregations among which are many polymorphonuclear leukocytes. At one point in the tissue, held in the interval between the artery ends, there is a deposit of lime salts suggesting the early stages of ossification. This sutured vessel demonstrates a favorable repair with a satisfactory reestablishment of the blood circulation (Fig. 2).

CONCLUSIONS.

1. Injuries of the abdominal aorta in dogs may be corrected with subsequent perfect restoration of the continuity of the vessel.

2. The complete occlusion of the aorta for a period of 30 minutes is not necessarily followed by serious consequences.

3. In cases in which a portion of the aorta must be resected, an arterial segment taken from another animal can be safely utilized as a transplant.

4. While the reestablishment of the continuity of the severed aorta by the circular suture is possible, the approximation of the severed ends during the suture entails such injury that thrombosis frequently occurs. Therefore, when the aorta is completely severed, the introduction of a transplanted segment is indicated.

5. An arterial tube of increased caliber made of smaller vessels such as the carotid lends itself readily as a transplant to the severed aorta, with a reasonable assurance of reestablishing the continuity of this vessel.

6. Defects in the aorta can be readily corrected by the use of fascial transplants with a minimum danger of thrombosis.

EXPLANATION OF PLATES.

Plate 23.

Fig. 1. Sutured vessel wall. *A*, suture line of vessel wall. Eversion of the coats of the vessel wall in order to bring the intima in contact with the intima at the suture line. There is a parietal coagulation of blood, *B*, into which reparative tissue grows in the final establishment of repair. Compare with Fig. 2, in which growth of tissue is complete, reestablishing the intimal lining of the vessel.

Plate 24.

Fig. 2. Sutured vessel wall. The everted vessel wall, *A*, joins the tissue transplant, *B*. The interval between the vessel wall and tissue transplant is covered by a mass of reparative tissue, *C*, which has grown into a blood coagulum, usually found present in early specimens after vascular suture. Compare with Fig. 1.

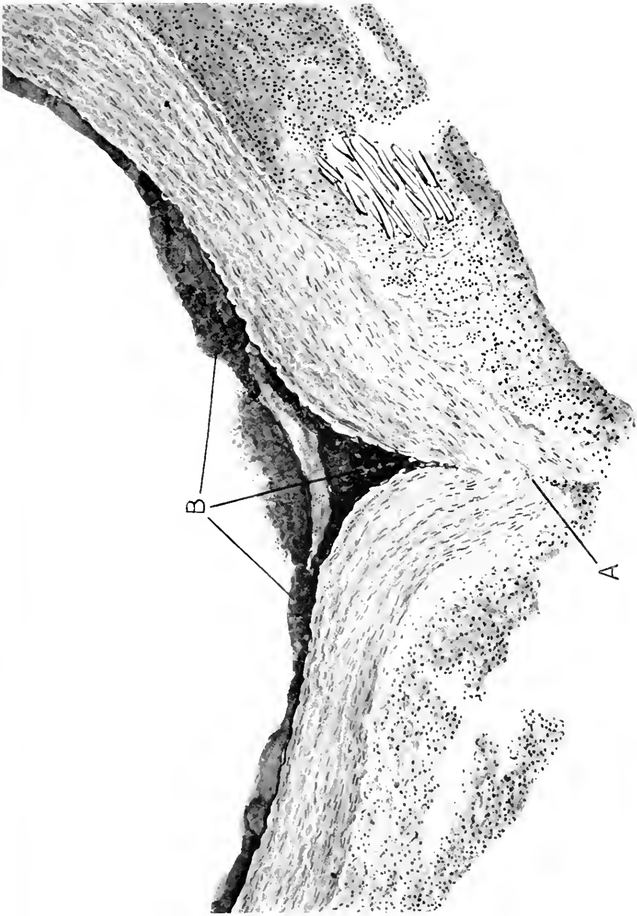


Fig. 1.

(Goodman: Operations on the abdominal aorta.)

Fig. 2.

(Goodman: Operations on the abdominal aorta.)

MORPHOLOGICAL CHARACTERISTICS AND NOMENCLATURE OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ (INADA AND IDO).

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Plates 25 to 29.

(Received for publication, February 27, 1918.)

In a previous communication, the writer reported the presence in American wild rats of a spirochete morphologically and immunologically identical with the *Spirochæta icterohæmorrhagiæ* of Inada and his associates and also with the strain isolated by Stokes from cases of infectious jaundice among British soldiers in Flanders.[1] The European strains, which have now been isolated from cases on the British, French, and Italian fronts, as well as from wild rodents captured not only near the battle-lines but in regions remote from them, are undoubtedly strains of the same organism.[2] Jobling and Eggstein[3] have also found the same spirochete recently among wild rats caught in Tennessee.

Just how, in nature, a rat becomes a carrier of the spirochete is not at once apparent. It is not improbable that the contamination of a foodstuff by the urine of an infected rat may transmit the organism to other rats; or the animal may become infected by feeding upon an infected dead rat, since a rat may be experimentally infected by feeding it with an infected foodstuff or with an infected tissue or

[1] Noguchi, H., *J. Exp. Med.*, 1917, xxv, 755.

[2] Costa, S., and Troisier, J., *Presse méd.*, 1916, lxxx, 526, 565. Courmont, J., and Durand, P., *Bull. et mém. Soc. méd. hôp.*, 1917, xli, series 3, 115. Clément, P., and Fiessinger, N., *Presse méd.*, 1916, lxxx, 598. Garnier, M., *Compt. rend. Soc. biol.*, 1916, lxxix, 928. Manine, Cristau, and Plazy, *Compt. rend. Soc. biol.*, 1917, lxxx, 531. Wilmaers, L., and Renaux, E., *Arch. méd. Belges*, 1917, lxx, 115, 207. Dawson, B., and Hume, W. E., *Quart. J. Med.*, 1916–17, x, 90. Zironi, A., and Capone, G., *Sperimentale*, 1917, lxxi, 298. Ascoli, M., and Perrier, S., *Gazz. osp.*, 1916, xxxvii, 1618. Sisto, P., *Sperimentale*, 1917, lxxi, 361. Siccardi, P. D., and Bompiani, G., *Ann. ig.*, 1917, xxvii, 609. Moreschi, C., *Policlinico, Sez. Prat.*, 1917, xxiv, 265. Sampietro, G., *Ann. ig.*, 1917, xxvii, 23.

[3] Jobling, J. W., and Eggstein, A. A., *J. Am. Med. Assn.*, 1917, lxix, 1787.

organ. Whatever the mode of preservation in nature, *Spirochæta icterohæmorrhagiæ* is a common commensal among rodents.

Morphology.

The morphology of this organism has been the subject of much study by its discoverers and by others, but its distinctive feature does not seem to have been recognized. Inada and his associates described the organism as a spirochete with several irregular waves, the entire body being dotted with alternate bright and shadowy portions.[4] Hübener and Reiter, who described a similar picture, apparently believed that the organism had a series of minute knots, and hence gave it the name *Spirochæta nodosa*.[5]

That these investigators overlooked the true structure must have been due either to the difficulty of observing the organism, even under a powerful dark-field illumination, or to the indistinctness of the minute spirals in a stained preparation. It appears as an almost smooth bodied, wavy organism, not unlike *Spironema refringens* when fixed in methyl alcohol and stained with Giemsa's solution (Figs. 1, 2, and 3). As has been said in a previous paper,[1] the natural features of the organism can be well preserved when it is fixed in osmic vapor and then stained over night with Giemsa's solution. In such a preparation it is stained light purple and is seen to consist of a very tightly and regularly wound cylindrical filament tapering to sharply pointed extremities. The filament usually assumes a graceful hook at one or both ends, while the main portion may be straight or slightly bent (Figs. 4 and 5). The number of spirals (not waves) varies considerably according to the length of the specimen, which may be between 3 to 20, 30, or even 40 μ, but the distance between the apices of two spirals measures about 0.5 μ. For example, a specimen measuring 9 μ would have eighteen spirals. The thickness, or diameter of a cross-section, of the organism is nearly uniform until it approaches the terminal portion, which may be so conveniently designated because of its tapering points and its hooked attitude. The number of spirals in the terminal portion appears to

[4] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[5] Hübener and Reiter, *Deutsch. med. Woch.*, 1916, xlii, I.

be about six in all specimens, and it is this portion which exhibits the greatest tendency to become bent to a semicircle. Unlike various spironemata or treponemata, the spiral amplitude near the extremities is not noticeably less than that of the middle portion of the organism.

In certain specimens the terminal portions are far less intensely stained than the main portion (Figs. 1, 2, and 3). In the majority of specimens, both terminal portions are bent to the same side (Figs. 5, 6, 13, 16, 17, 19, and 22), but in some they form hooks of opposite direction (Fig. 4), unipolar hooks (Figs. 8, 9, 15, and 20), or are not bent at all (Figs. 11 and 12); and some are contorted (Fig. 18). In the less well preserved specimens the spirals are no longer distinct but appear as somewhat more deeply stained dots (Fig. 21). As has already been pointed out, under a powerful dark-field illumination the organism in rapid rotary motion seems to be surrounded by a halo. This may be only an optical effect, but a similar clear zone has been noticed in the stained preparations of some specimens (Figs. 12, 15, 17, and 23).

The dark-field picture of the organism is such that one may mistake the minute spirals for refractive beads arranged diagonally or somewhat obliquely with respect to the axis of the organism (Figs. 24 and 27 to 33), as originally depicted by Inada and his associates and others. But, as has been stated before, with a favorable and powerful illumination, the real structure can be revealed (Figs. 24, 25, and 34).

Only a few of the photomicrographs represent the characteristically hooked forms (Figs. 26 and 28) as actually seen in active rapid rotary motion in a free space, because it was difficult to photograph the organisms in motion, and as soon as motion ceases many of them lose the typical hooks. The large wavy undulations, however, (not the elementary spirals), as assumed by the organisms when penetrating semifluid medium, are well shown in some of the specimens at rest (Figs. 27 and 29 to 33). The remarkable flexibility of the organism in a semisolid medium is also shown (Figs. 27, 32, and 33). These minute filamental organisms dart through the soft medium with great rapidity, first in one direction and then in another, searching for a loose spot which they can pierce through. When encountering

an impenetrable obstacle they reverse their progression and start anew. A striking sight is thus presented by these little vermicular organisms darting in all directions. A vibratory motion of the free portion of the organism results when it is extricating itself from an entanglement. In an emulsion of infected liver one may encounter a tangle of several actively motile organisms (Figs. 24 and 25), while in a culture several weeks old a mass of hundreds of motile spirochetes may be found (Fig. 35).

The European (Figs. 36 to 57) and Japanese (Figs. 58 to 68) strains have all the morphological features given for the American strain. It might be mentioned here that the elementary spirals in the terminal portion are much smaller in number and less regular in the stained specimens of the European strain, but this may be due to imperfect fixation of the organism, because under the dark-field microscope the spirals are equally close and regular.

Classification.

Characteristics of Different Genera of Spiral Organisms.

In order to determine the systematic position of the organism of infectious jaundice, it may be well to review here the characteristics of various genera of spiral organisms. Through the recent investigations of Gross,[6] Zuelzer,[7] Dobell,[8] Gonder,[9] Swellengrebel,[10] and others, the organism for which Ehrenberg created the term *Spirochæta* in 1838 is now known to be distinct from the majority of so called spirochetes. It consists of a long, highly flexible, central axial filament surrounded by a regularly wound layer of protoplasm, usually of great length (200 to 500 μ), and is free living in fresh or marine water (Fig. 108). Neither a membrane nor a flagellum is present. Multiplication takes place by transverse fission. The organism

[6] Gross, J., *Centr. Bakteriol., 1te Abt., Orig.*, 1912, lxv, 83.

[7] Zuelzer, M., *Arch. Protistenk.*, 1912, xxiv, 1.

[8] Dobell, C., *Proc. Roy. Soc. London, Series B*, 1912, lxxxv, 186.

[9] Gonder, R., Spironemacea (Spirochaeten), in von Prowazek, S., Handbuch der pathogenen Protozoen, Leipsic, Liefg. 6, 1914, 671.

[10] Swellengrebel, N. H., *Ann. Inst. Pasteur*, 1907, xxi, 448; *Compt. rend. Soc. biol.*, 1907, lxii, 213.

creeps along the surface of an object but does not swim. Only four species belonging to this genus have been described. The organism under discussion does not belong to it.

Cristispira and Saprospira.—For a limited variety of coarse, actively motile spiral organisms infesting the crystalline styles of certain mollusca, the genus *Cristispira* was proposed by Gross in 1910.[11] The characteristic features are: the presence of a membranous structure running spirally from one end of the body to the other, assuming the aspect of a crista or ridge; the chambered structure of the body; the absence of a terminal filament; and the existence of a strong, flexible membrane (Figs. 104 to 106). According to Gross, reproduction may be effected by multiple transverse fission or sporulation, though I have failed to confirm the occurrence of sporulation. More than a dozen species have been described, but from personal observations I doubt whether these so called species are sufficiently characteristic to be so distinguished. The type organism was first described by Certes in 1882[12] as found in oysters, and was known as *Spirochæta* or *Trypanosoma balbianii*. Another genus, *Saprospira*, was proposed by Gross in 1912[13] for a few varieties of spiral organisms in mussels which differed from the cristispiræ in not having a crista (Fig. 107). The organism in question, however, belongs to neither of these genera.

Spironema and Treponema.—Next in order is the large group of small parasitic spiral organisms commonly called spirochetes. Among them are the causative agents of syphilis and yaws (Figs. 69 to 72 and 103) and of relapsing fevers in man and animals (Figs. 94 to 100), non-pathogenic parasites in certain rodents, and various saprophytic types on or about the oral, alimentary, or genital mucous membranes (Figs. 73 to 93). Their essential feature is a spiral flexible body with terminal filaments, but no undulating membrane. They seem to multiply by transverse as well as longitudinal fission. The rigidity of the curves differs greatly in different organisms, some becoming almost flat at death or constantly changing the waves by oscillatory undulation, others retaining their regular curves even during motion or after death. The whole group has been called *Spirochætæ* or *Spirilla*, in spite of the

[11] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 41.

[12] Certes, A., *Bull. Soc. zool. franc.*, 1882, vii, 347; 1891, xvi, 95.

[13] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 188.

fact that they have no affinity with the real spirochete or non-flexible spirillum. Gross includes them in the genus *Spironema*, a term introduced by Vuillemin[14] in 1905 to distinguish Schaudinn's organism of syphilis from those with less rigid spirals. Dobell,[8] however, believes that the term *Treponema*, as proposed by Schaudinn[15] himself in 1905 for his organism, should be employed to designate all these minute parasitic varieties. Gonder[9] takes a more conservative stand and upholds the distinction made by Schaudinn between the treponema type and that with less constant curves. For example, Gonder retains the genus *Spironema* for the latter and *Treponema* for the former type. I agree with Gonder in this respect, as the general features are sufficiently distinct to enable one to differentiate the two groups.

Nomenclature of Leptospira (Spirochæta) icterohæmorrhagiæ.

The striking differences between the organism of infectious jaundice and all the other so called spirochetes, or rather spironemata and treponemata, are apparent at a glance. The closely set, regular spirals of the organism of Inada and Ido remain unmodified during its rotary, spinning motions in a free space and when it is piercing a semisolid medium. While in motion in a free space, the whole body appears tightly drawn into a straight line, except for the usual hook formation of one or both terminal portions. When one end is extended and straight and the other semicircularly hooked, the organism usually progresses in the direction of the straight portion and seems to be propelled from the rear by the rotating hook (Figs. 8, 9, 15, and 20). A specimen with both ends hooked remains stationary in spite of its rapid rotary motions (Figs. 13, 16, and 19). By straightening one end or the other alternately, the organism changes its progression from one direction to the opposite one. When the organism penetrates a soft medium, changing direction very rapidly, it seldom shows hooked ends (Figs. 11, 12, 29, 30, 32, and 33). In this sort of movement the body assumes wide wavy undulations such as are seen in an active specimen of *Spironema refringens*. The behavior of the organism in semisolid medium is different from that

[14] Vuillemin, P., *Compt. rend. Acad.*, 1905, cxl, 1567.

[15] Schaudinn, F., *Deutsch. med. Woch.*, 1905, xliii, 1728.

in a free space. The persistence of the minute elementary spirals at all times is a feature which distinguishes this organism from any treponema or spironema. The depth of the spirals does not exceed the diameter of the body, a fact unknown among other so called spirochetes. A diligent search by means of various staining methods, as well as by dark-field illumination applied to cultures of different ages, has not demonstrated a terminal flagellum or peritrichal flagella or membranes. It is well to recall at this point that in old cultures of all the spironemata or treponemata I have isolated I have been able to demonstrate the presence of a terminal filament, even when it was observed with difficulty in uncultivated specimens. This organism, however, as far as we know at present, moves by means of its terminal portions. Moreover, unlike spironemata and treponemata, it withstands the action of 10 per cent saponin. Clearly it does not belong to either of these genera, but must remain in a class by itself until other similar organisms come to our observation. The nearest approach to it in morphological and biological respects is *Spirochæta biflexa*, which was isolated by Wolbach and Binger[16] in 1914 from a filtrate of stagnant water taken from the shore of a fresh water pond near Boston. There is a great similarity between the two organisms. Both are filterable through Berkefeld filters. Wolbach and Binger did not succeed in obtaining a second generation in culture, and no tests of pathogenicity for experimental animals were made.

For the reasons which have been discussed, it seems justifiable to include the type of organism in question under *Leptospira* (λεπτός fine, + σπεῖρα, coil), as has already been proposed.[1]

The genera with their type organisms are presented below. The measurements of each of these representative members and the characteristic features used for identification of the genera are considered. There is little difficulty in distinguishing *Spirochæta*, *Saprospira*, *Cristispira*, and *Leptospira* from one another. But the distinction between *Spironema* and *Treponema* depends chiefly upon the rigidity and regularity of the spirals which are characteristic of the treponemata. Under natural conditions this difference is so marked that there should be no confusion in classification, but under cultural

[16] Wolbach, S. B., and Binger, C. A. L., *J. Med. Research*, 1914, xxx, 23.

CORRECTION.

On page 583, Vol. xxvii, No. 5, May 1, 1918, the scale of Text-fig. 1 should read 0, 5, 10, and 15 μ instead of 0, 10, 20, and 30 μ.

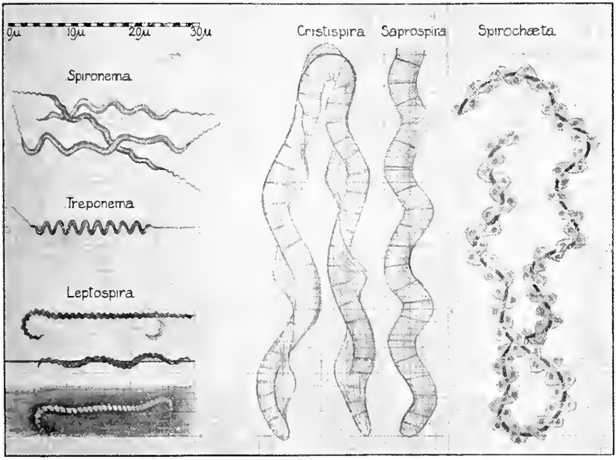
to the morphological modifications due to cultivation, regard distinction between *Treponema* and *Spironema* as insufficient to maintain two separate genera, and Dobell chose the term *Treponema* and Gross *Spironema* for the same group of organisms. In my opinion the characteristics of *Treponema* and *Spironema*, under natural conditions, are sufficiently pronounced to justify retaining the two terms in classification. Neither *Treponema* nor *Spironema* has any feature which is likely to be confused with those of the other four genera referred to above. Text-fig. 1 shows the types mentioned below.

Genus.—Spirochæta (Ehrenberg, 1838). *Type Organism.—Spirochæta plicatilis* (Ehrenberg, 1838) (Fig. 108). *Measurements.*—Length, 100 to 500 μ; blunt end. Diameter, 0.5 to 0.75μ; cylindrical. Spiral amplitude, 2 μ; regular. Spiral depth, 1.5 μ; regular. Waves, several, large, inconstant, irregular. *Axial Filament.*—Distinct in stained specimens; flexible; elastic. *Chambered Structure.*—Absent. *Membrane.*—Absent. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in fresh or marine water. *Other Species.—Plicatilis marina, plicatilis eustrepta, stenostrepta, daxensis. Staining Properties of Axial Filament and Cell Membrane.*—Axial filament consists of chitin or cutin-like substance. Stains violet by Giemsa's solution and gray by iron-hematoxylin. *Staining Properties of Body.*—Plasmic spirals of the body stain with eosin, rubin, etc. Contain volutin granules. *Trypsin Digestion.*—Axial filament resistant. *Bile Salts (10 Per Cent).*—Becomes shadowy pale but is not dissolved. *Saponin (10 Per Cent).*—Lives 30 minutes. Later becomes shadowy, but is not dissolved.

Genus.—Saprospira (Gross, 1911). *Type Organism.—Saprospira grandis* (Gross, 1911). *Measurements.*—Length, 100 to 120 μ; obtuse end. Diameter, ?μ; cylindrical. Waves, large, inconstant, shallow, irregular, 3 to 5 in number. Sometimes almost straight. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.*—Distinct, flexible, elastic. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in foraminiferous sand. *Other Species.—Nana.*

Genus.—Cristispira (Gross, 1910). *Type Organism.—Cristispira balbianii* (Certes, 1882) (Figs. 104 and 105). *Measurements.*—Length, 45 to 90 μ; obtuse end. In stained preparations the end may be sharply pointed, but this is due

to shrinkage by fixing reagents. Diameter, 1 to 1.5 μ; cylindrical. Waves, 2 to 5, sometimes more, large, irregular, shallow. In a dying specimen the waves may be more numerous and regular. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.* Distinct, flexible, elastic. *Crista.*—Present, a ridge-like membrane. Spirally wound body. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Parasitic in the alimentary canals of shell-fish. *Other Species.*—*Ostræ, anodontæ, modiolæ, veneris, tapetos, chamæ*, etc. *Staining Prop-*



TEXT-FIG. 1. Diagram contrasting the characteristic features and relative proportions of *Spironema, Treponema, Cristispira, Saprospira, Spirochæta*, and *Leptospira*. The scale in microns is given in the upper left-hand corner of the figure.

erties of Axial Filament and Cell Membrane.—Membrane behaves like chitin or cutin substance. Stains violet by Giemsa's solution and light gray by iron-hematoxylin. *Staining Properties of Body.*—The body is alternately stained red and bluish violet and the crista red by Giemsa's solution. Iron-hematoxylin brings out sharp septa and a layer of chromatin granules. *Trypsin Digestion.*—Membrane resistant. Crista and chambers disappear. *Bile Salts (10 Per Cent).* Crista quickly destroyed. Body not attacked. *Saponin (10 Per Cent).*—Crista becomes fibrillar, then indistinct. Body not affected.

Genus.—Spironema (Vuillemin, 1905). *Type Organism.—Spironema recurrentis* (Lebert, 1874[17]) (Figs. 94 to 96). *Measurements.*—Length, 8 to 16 μ; pointed ends. Diameter, 0.35 to 0.5 μ; cylindrical or slightly flattened. Spirals, large, wavy, inconstant, about five in number. Closer and more regular in cultures. *Axial Filament.*—Probably present. *Chambered Structure.*—Absent. *Membrane.*—Delicate, flexible, double contoured. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present, easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse, possibly also longitudinal. *Habitat of Genus.*—Numerous pathogenic and non-pathogenic varieties. *Other Species.*—*Carteri, kochi, novyi, duttoni, berbera, aegyptica, gallinarum, anserina, theileri, equi, muris, eugyratum, microgyratum, buccalis, refringens,* etc. *Staining Properties of Axial Filament and Cell Membrane.*—Membrane difficult to differentiate. *Staining Properties of Body.*—Stains violet by Giemsa's solution. *Bile Salts (10 Per Cent).*—Disintegration complete. *Saponin (10 Per Cent).*—Immobilized in 30 minutes then broken up in a few hours. In some there is an axial filament laid bare.

Genus.—Treponema (Schaudinn, 1905). *Type Organism.—Treponema pallidum* (Schaudinn and Hoffmann, 1905[18]) (Figs. 69 to 72 and 103). *Measurements.*—Length, 6 to 14 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 1 μ; regular, rigid. Spiral depth, 0.8 to 1 μ; very constant. Waves, one or more slight undulating curves may be present. *Axial Filament.*—Doubtful. The whole seems to consist of a spirally wound axial filament. *Chambered Structure.*—Absent. *Membrane.*—Doubtful; if there is one it must be flexible. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present. Easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse or possibly also longitudinal. *Habitat of Genus.*—Two pathogenic and several harmless parasites. *Other Species.*—*Pertenue, microdentium, macrodentium, mucosum, calligyrum, minutum.* *Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains pink by Giemsa's solution. *Trypsin Digestion.*—Resists digestion for many days. *Bile Salts (10 Per Cent).*—Disintegration complete. *Saponin (10 Per Cent).*—Broken up in time.

Genus.—Leptospira (Noguchi, 1917). *Type Organism.—Leptospira icterohæmorrhagiæ* (Inada and Ido, 1914) (Figs. 1 to 68, 101, and 102). *Measurements.*—Length, 7 to 9 to 14 μ; exceptionally 30 to 40 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 0.45 to 0.5 μ; regular, rigid. Spiral depth, 0.3 μ; regular. Waves, one or more gentle wavy curves throughout the entire length. When in a free space, one or both ends may be semicircularly hooked, while in semisolid media the organism appears serpentine, waved, or bent. Its flexibility is most striking. *Axial Filament.*—Not recognized. *Chambered Struc-*

[17] Lebert, H., Rückfallstyphus, Flecktyphus und Cholera, in von Ziemssen, H., Handbuch der speciellen Pathologie und Therapie, Leipsic, 1874, ii, 267.

[18] Schaudinn, F., and Hoffmann, E., *Arb. k. Gsndhtsamte.*, 1905, xxii, 527.

ture.—Absent. *Membrane.*—Not recognized. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Not recognized. *Flagella.*—Absent. *Highly Motile End Portion.*—Well developed in the last six to eight spirals. *Division.*—Transverse. *Habitat of Genus.*—One pathogenic and one possibly non-pathogenic variety known. *Other Species.*—*Biflexa* (Wolbach and Binger). *Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains reddish violet by Giemsa's solution. *Bile Salts (10 Per Cent).*—Easily dissolved. *Saponin (10 Per Cent).*—Completely resistant.

The comparative dimensions of these representative organisms may be shown by putting side by side the diameter, spiral amplitude, spiral depth, and length of each, taking the diameter of the finest member, *Leptospira icterohæmorrhagiæ*, as a unit of comparison (Table I).

TABLE I.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1.2 (0.3 μ)	56 (14 μ)
Treponema pallidum..........	1.2 (0.3 μ)	4 (1 μ)	3.6 (0.9 μ)	48 (12 μ)
Spironema obermeieri..........	2 (0.5 μ)	12 (3 μ)	6 (1.5 μ)	32 (8 μ)
Cristispira balbianii...........	5 (1.2 μ)	60 (15 μ)	24 (6 μ)	200 (50 μ)
Saprospira grandis............	5 (1.2 μ)	32 (8 μ)	8 (2 μ)	400 (100 μ)
Spirochæta plicatilis...........	3 (0.75 μ)	18 (4.5 μ)	6 (1.5 μ)	600 (150 μ)

One may obtain the comparative proportions for each genus by using the diameter of its representative member as a unit of comparison, as in Table II.

TABLE II.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Spirochæta plicatilis...........	1 (0.75 μ)	6 (4.5 μ)	2 (1.5 μ)	200 (150 μ)
Saprospira grandis............	1 (1.2 μ)	7 (8 μ)	1.8 (2 μ)	83 (100 μ)
Cristispira balbianii...........	1 (1.2 μ)	13 (15 μ)	5 (6 μ)	41 (50 μ)
Spironema obermeieri..........	1 (0.5 μ)	6 (3 μ)	3 (1.5 μ)	16 (8 μ)
Treponema pallidum..........	1 (0.3 μ)	3.3 (1 μ)	3 (0.9 μ)	40 (12 μ)
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1 (0.25 μ)	56 (14 μ)

The proportions are distinctive for each genus, and form, with other differentiating features already discussed, a fairly well established basis for the classification of these spiral organisms, hitherto so indis-

criminately called by the general name of spirochetes. It would be desirable, in describing a new spiral organism, to place it in one of the six classes discussed, since under the vague name of spirochete no one can visualize the actual features of the organism in question, while if it is called *Leptospira*, for example, certain definite features are connoted, and confusion with other so called spirochetes is avoided. This is particularly important when one is examining specimens of urine such as those from certain cases in which a *Leptospira* or a *Treponema* may be present alone or together, as in a study of trench infections. Patterson[19] and Nankivell and Sundell[20] discovered the latter type in cases of trench fever of unknown origin, while the former has been found responsible for a number of cases of various trench affections.[21,22]

A brief note may be made of the relation of *Leptospira* to a comparatively minute species of spirochete, *Spirochæta stenostrepta*, described by Zuelzer[7] (Figs. 109 and 110). The organism was found in stagnant water with *Spirochæta plicatilis*. It has a diameter of 0.25 μ and a length of 20 to 60 μ, seldom reaching a length of 200 μ. In a short specimen which measured 13 μ there were eleven spirals. In life an axial filament was recognized. Here the leptospira can be distinguished by its lack of an axial filament and its closer spirals. In the latter respect certain oscillatorial organisms such as *Spirulina vesicolor* (Figs. 111 and 112), or *Spirulina tenuissima* have a superficial resemblance to leptospira, but their multicellular structure, which can be demonstrated by subjecting them to a preliminary treatment with trypsin solution before staining, shows them to be very different. Each coil here represents an individual cell separated from the adjoining cells by walls. The spirulina has blunt ends and does not exhibit the active, brusque movements characteristic of leptospira.

[19] Patterson, S. W., *J. Roy. Army Med. Corps*, 1917, xxix, 503.

[20] Nankivell, A. T., and Sundell, C. E., *Lancet*, 1917, ii, 672, 836.

[21] Couvy, L., and Dujarric, R., *Compt. rend. Soc. biol.*, 1918, lxxxi, 22.

[22] Dudgeon, L. S., *Lancet*, 1917, ii, 823.

SUMMARY.

The present study deals with the morphology and systematic position of the causative agent of infectious jaundice. There are several features which are not found in any of the hitherto known genera of Spirochætoidea which led me to give this organism an independent generic name, *Leptospira*, denoting the peculiar minute elementary spirals running throughout the body. The absence of a definite terminal flagellum or any flagella, and the remarkable flexibility of the terminal or caudal portion of the organism are other distinguishing features. Unlike all other so called spirochetes the present organism resists the destructive action of 10 per cent saponin.

A detailed comparative study of related genera, including *Spirochæta*, *Saprospira*, *Cristispira*, *Spironema*, and *Treponema*, has been given with the view of bringing out more strongly the contrast between them and the new genus.

A study has been made to discover whether any differential features exist among the strains of *Leptospira icterohæmorrhagiæ* derived from the American, Japanese, and European sources, but none has been found.

It is hoped that the creation of a new genus may facilitate a more exact morphological description than has hitherto been possible, due to the vague use of the term *Spirochæta* which indiscriminately covered at least six large genera of spiral organisms.

EXPLANATION OF PLATES.

Plate 25.

Figs. 1 to 23 show the morphological features of the American strain of *Leptospira icterohæmorrhagiæ* in stained preparations.

Fig. 1. *Leptospira icterohæmorrhagiæ* in the blood of an experimentally infected guinea pig, showing irregular refringent waves, but no minute elementary spirals. Methyl alcohol fixation and Giemsa's solution. × 1,000.

Fig. 2. The same in a liver emulsion from a similar animal. Except for the few moderate undulations of the body, there is no indication here that these are spiral organisms. Methyl alcohol fixation and Giemsa's solution. × 1,000.

Fig. 3. The same in a kidney emulsion. Fixation and staining the same as above. × 1,000.

FIG. 4. The same in a blood specimen of an infected guinea pig. Fixation and staining the same as above. × 1,000.

Figs. 1 to 4 are intended to show the appearance of the leptospiræ in an air-dried specimen, fixed with methyl alcohol, and stained with Giemsa's solution. They do not show any elementary spirals and appear as smooth, somewhat wavy filaments.

FIGS. 5 to 11. *Leptospira icterohæmorrhagiæ* in stained preparations from a culture in its first generation on the 5th day. They were fixed when moist by osmic acid vapor for 2 minutes, then hardened in absolute alcohol for 30 minutes, and after being thoroughly washed in distilled water, were stained over night with Giemsa's solution (1:20 dilution). In these preparations there were many instances where the fixation and staining were not so satisfactory as in the specimens shown in these photomicrographs. A careful examination makes possible recognition of the closely set, minute, regular spirals throughout the entire length of the organism. With a magnification of 1,000 they are almost too minute to enable one to count the number of the spirals. × 1,000.

FIGS. 12 to 23. *Leptospira icterohæmorrhagiæ* magnified 3,000 times, which brings out the features more distinctly. All except Figs. 21 to 23 show the elementary spirals well. There are ten to twelve spirals to every 5 μ, making the distance between the apex of one spiral to that of the next about 0.5 μ. The terminal portions of the organisms are recognized by the gradually decreasing diameter and the coloration, which is lighter than that of the main portion of the body. These end portions seem to possess about six elementary spirals and measure about 3μ in length. They exhibit remarkable activity and flexibility and serve as propellers in progression in free space and as feelers in guiding the organism through a semisolid medium. Note Fig. 12.

Fig. 18 shows a specimen fixed probably during a somersault movement. The elementary spirals appear as dimly stained cross bars (imperfect fixation).

Fig. 21 shows three organisms attaching themselves to a red corpuscle. The spirals are not distinctly brought out, but one recognizes them as more intensely stained dots, arranged obliquely with respect to the optical axis of the organism.

Fig. 22 (also Figs. 13 and 19) shows a specimen fixed while rotating on its axis in a free space. The organism was otherwise stationary, as shown by its symmetrically bent hooks. Compare with Fig. 20, which has one hook, and therefore must be proceeding in the direction of the straight end.

The two specimens in Fig. 23 show no definite direction of progression. The spirals, though not well fixed, are fixed sufficiently for recognition.

In Figs. 12, 15, 17, 21, and 23, there is a clear space, or halo (about 0.15 μ wide) about the organisms along the entire length. Whether this clear zone, or halo, indicates the presence of a less chromatic membrane enveloping the organisms or is merely due to the dispersion of particles (culture media) from their immediate neighborhood by their rotary movements cannot yet be determined.

Plate 26.

Figs. 24 to 35. Specimens of the American strain of *Leptospira icterohæmorrhagiæ* as seen under the dark-field microscope.

Fig. 24. The organisms in a liver emulsion of an experimentally infected guinea pig. They are in resting position and show no characteristic hooked ends. One isolated leptospira has both ends hooked, but not typically, as it would be while actively rotating or progressing in a free space. The spirals appear as regularly set cross bands. × 1,000.

Figs. 25 and 26. A higher magnification of the same specimens. The finely set regular spirals are distinctly shown at the right in Fig. 24, and the cross-barred or dotted aspect of the spirals is shown in the other two of the same figure and also in Fig. 26. × 3,000.

Fig. 27 (× 1,000) and Figs. 28, 29, and 30 (× 3,000) show the leptospiræ in the kidney emulsion of an infected guinea pig. Except for the specimen at the center of Fig. 27, the organisms are in undulatory positions, with gracefully wound, rather loose waves. This position almost always indicates that the organisms are in a semisolid medium, which they are penetrating by means of spiral propulsion. They often remain in the same position for some time before renewing their efforts to extricate themselves. Their dotted or cross-barred appearance remains unmodified under these circumstances.

Fig. 31 (× 1,000) and Fig. 32 (× 3,000) show similar but more pronounced characteristic features.

Fig. 34. The minute elementary spirals are plainly seen in the three entangled leptospiræ in the right upper corner, while in three organisms of Fig. 33 they are recognizable only as dots or bars. × 3,000.

Fig. 35. A large mass of leptospiræ in a fluid culture 3 weeks old. They grow considerably longer in such a medium and form a mass of entangled organisms having the same minute elementary spirals as uncultivated specimens. × 1,000.

Plate 27.

Figs. 36 to 57 represent the British strain (Stokes) of *Leptospira icterohæmorrhagiæ.*

Fig. 36. A Fontana preparation of the leptospiræ in the liver emulsion of an infected guinea pig. The elementary spirals can hardly be distinguished. × 1,000.

Fig. 37. A badly fixed osmic acid-Giemsa preparation, in which one of the organisms on the extreme right appears as a negative image with minute elementary spirals well brought out. The dye settled about the leptospira without staining the organism itself. × 1,000.

Figs. 38 to 43. A preparation better fixed with osmic acid vapor and well stained with Giemsa's solution. The leptospiræ were cultivated 7 days at 28°C. In none of them is there any difficulty in discerning the individual elementary

spirals throughout the entire length of the organism. Perhaps owing to imperfect fixation, the elementary spirals in the terminal portions are less numerous and the spiral depth is shallower than in the main portion, which also takes on a more intense stain. In the majority of specimens the spiral amplitude of the main portion is about the same as that of the American or the Japanese strain (0.5 μ). There are a few specimens, however, which measure 0.6 μ from one spiral to the next. $\times$ 1,000.

FIGS. 44 to 52. The same. $\times$ 3,000.

FIGS. 53 to 57. Dark-field views of the leptospira. Figs. 53, 56, and 57 are from a fluid medium, and Figs. 54 and 55 from a semisolid medium. $\times$ 1,000.

PLATE 28.

FIGS. 58 to 68. Dark-field views of the Japanese strain of *Leptospira icterohæmorrhagiæ* from a 7 day culture on semisolid medium. Figs. 58 to 62 are magnified 1,000 times and Figs. 63 to 68, 3,000 times. These photographs show the remarkable flexibility of the tight, elementary spirals of the organisms. The numerous circularly coiled specimens suggest the peculiar hoop-like coiling form of some specimens of *Cristispira balbianii* in the crystalline styles of oysters.

FIGS. 69 to 72. Dark-field views of *Treponema pallidum* which are given here for comparison with the leptospiræ. Their larger spiral amplitude and spiral depth, and their rigidity are sufficiently differentiating. Figs. 69 and 71 are magnified 1,000 times, and Figs. 70 and 72, 3,000 times.

PLATE 29.

Some of these photomicrographs are from stained and some from dark-field preparations. They are reproduced here to illustrate the differential characteristics of several constituent genera of the family of Spirochætoidea (Dobell). $\times$ 1,000.

Treponema Group.

FIGS. 73 to 76. Dark-field views of a minute treponema (*Treponema minutum, n. sp.*) found in a smegma. Their average spiral amplitude is 0.9 to 1 μ, spiral depth, 0.2 to 0.5 μ, average number of spirals, eight to ten in 7 to 9 μ, and thickness, 0.3 μ.

FIGS. 80 to 83. *Treponema calligyrum* in smegma. Spiral amplitude, 1.75 μ, depth, 0.5 to 1 μ, four to seven spirals in 7 to 12 μ, thickness, 0.4 to 0.5 μ.

FIG. 84. The same from a culture.

FIG. 85. *Treponema microdentium* from the mouth.

FIG. 86. The same from a culture.

FIG. 88. *Treponema macrodentium* from a culture.

FIGS. 92 and 93. A treponema from the urine of a child, resembling the smallest smegma treponema (Figs. 73 to 76).

Spironema Group.

FIGS. 77 and 78. *Spironema refringens* from smegma. Spiral amplitude, 2 to 2.75 μ; spiral depth, 0.5 to 1.5 μ; four to eight spirals in 11 to 16 μ; thickness, 0.7 μ.

FIG. 79. The same from a culture.

FIG. 87. *Spironema vincenti* from the mouth.

FIGS. 89 to 91. *Spironema buccalis* from the mouth. Spiral amplitude, 2.75 to 3.7 μ; spiral depth, 0.7 to 1 μ; four to seven and one-half spirals in 11 to 17 μ; thickness, 0.5 to 1 μ.

FIGS. 94 and 96. *Spironema recurrentis* in a culture.

FIG. 95. The same in the blood of an infected mouse.

FIG. 97. *Spironema duttoni* in a culture.

FIG. 98. *Spironema kochi* in a culture.

FIG. 99. *Spironema gallinarum* in a culture.

FIG. 100. *Spironema novyi* in the blood of an infected rat.

Leptospira Group.

FIG. 101. *Leptospira icterohæmorrhagiæ*, American strain. $\times$ 1,000.

FIG. 102. *Leptospira icterohæmorrhagiæ*, Japanese strain. $\times$ 1,000.

FIG. 103. *Treponema pallidum* for comparison. Same magnification.

Cristispira Group.

FIG. 104. Dark-field view of *Cristispira balbianii* from oysters obtained near Woods Hole.

FIG. 105. The same. Osmic acid fixation. Stained with Giemsa's solution.

FIG. 106. *Cristispira veneris* (?) from clams obtained near Long Island Sound. Sublimate alcohol fixation and Heidenhain's iron-hematoxylin.

Saprospira Group.

FIG. 107. An organism possibly belonging to this genus. It was cultivated by me from oysters obtained near New York.

Spirochæta Group.

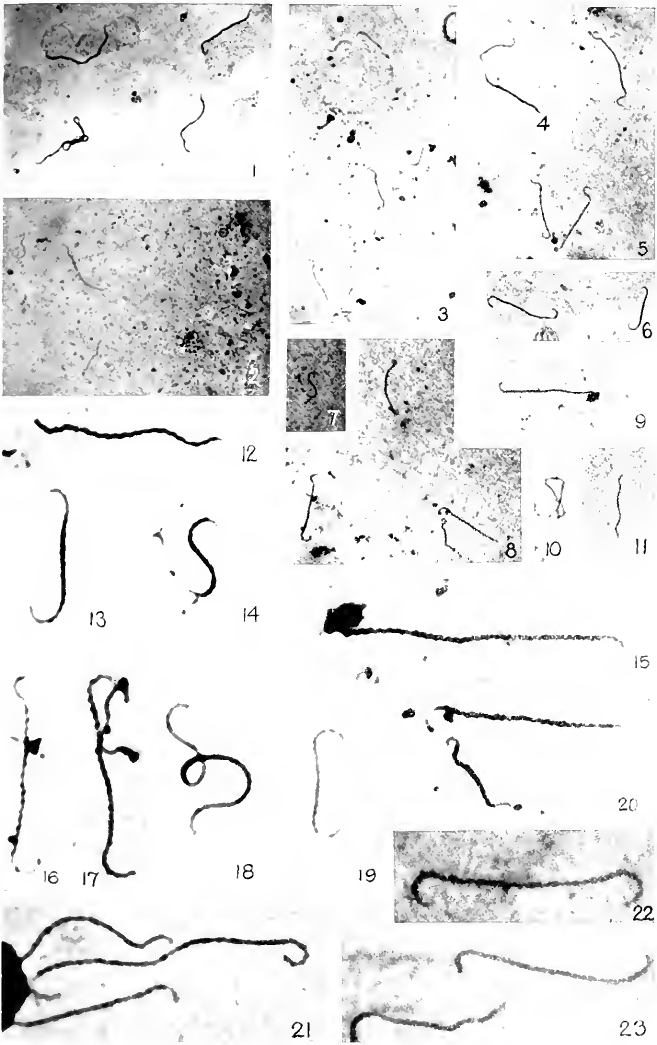
FIG. 108. *Spirochæta plicatilis*. Sublimate acetic-acid-alcohol fixation and iron-hematoxylin (after Zuelzer).

FIGS. 109 and 110. *Spirochæta stenostrepta* (after Zuelzer).

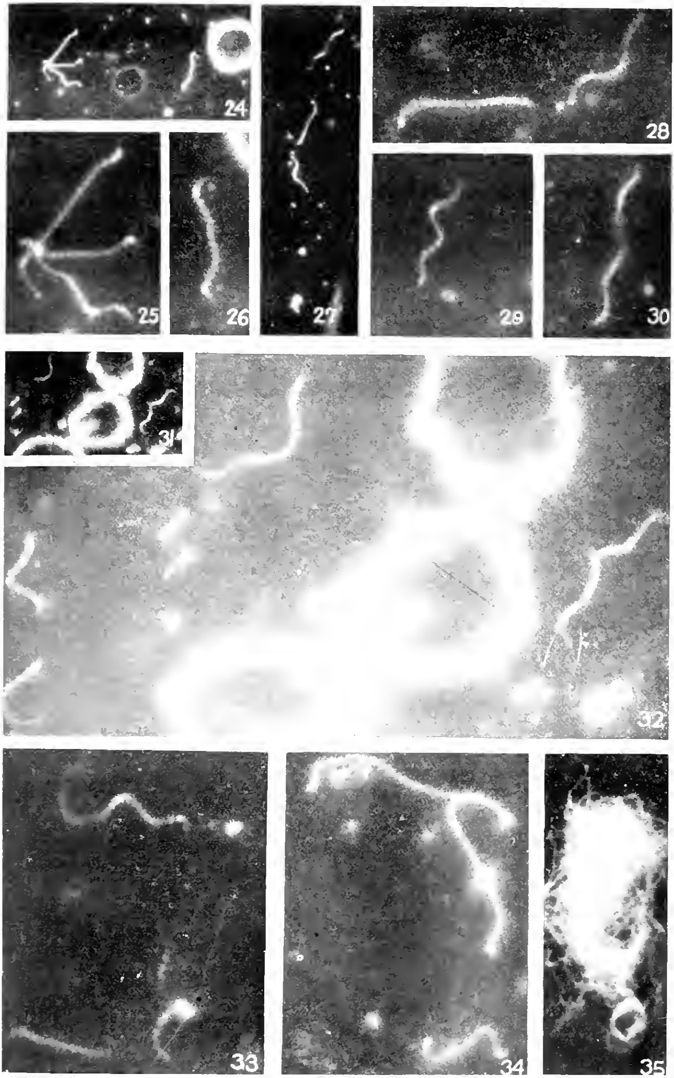
Spirulina Group.

Fig. 111. *Spirulina versicolor.* This organism does not belong to the family of Spirochætoidea, but on account of its close spirals it is shown here. Iodine-alcohol and Delafield hematoxylin (after Zuelzer).

Fig. 112. The same, at another plane of focus, where the innermost structure is not brought out as in Fig. 111.



Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.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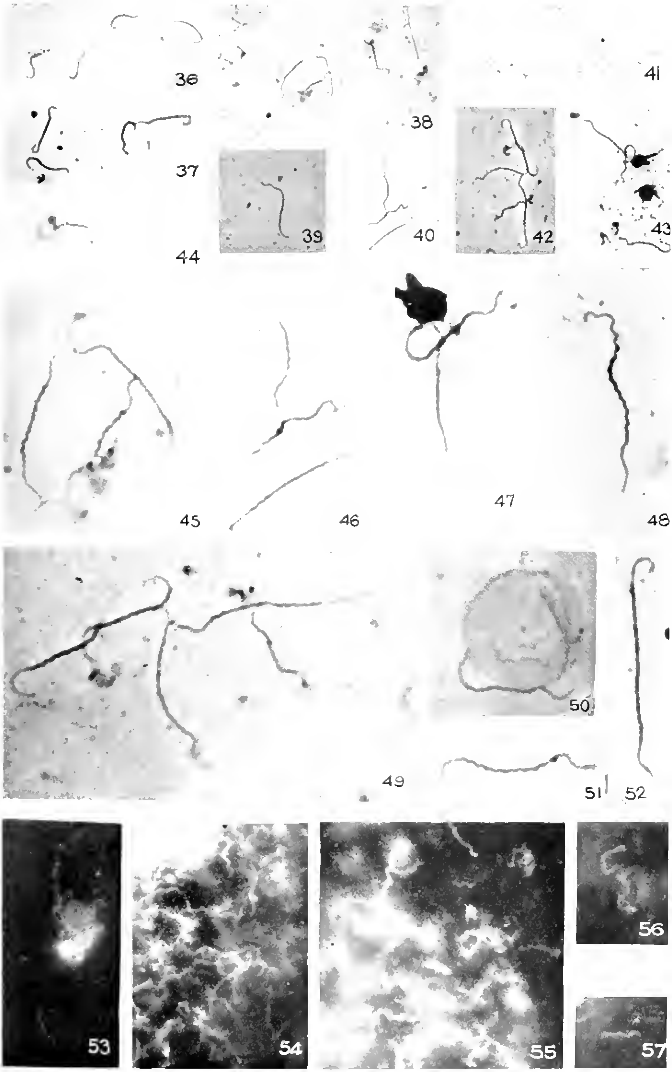
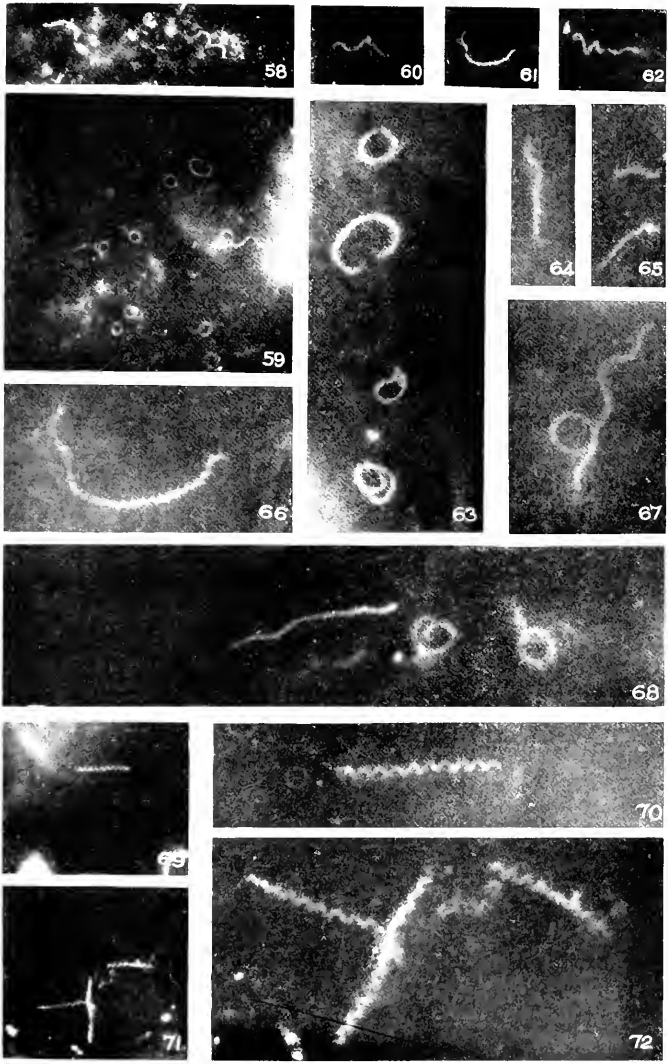
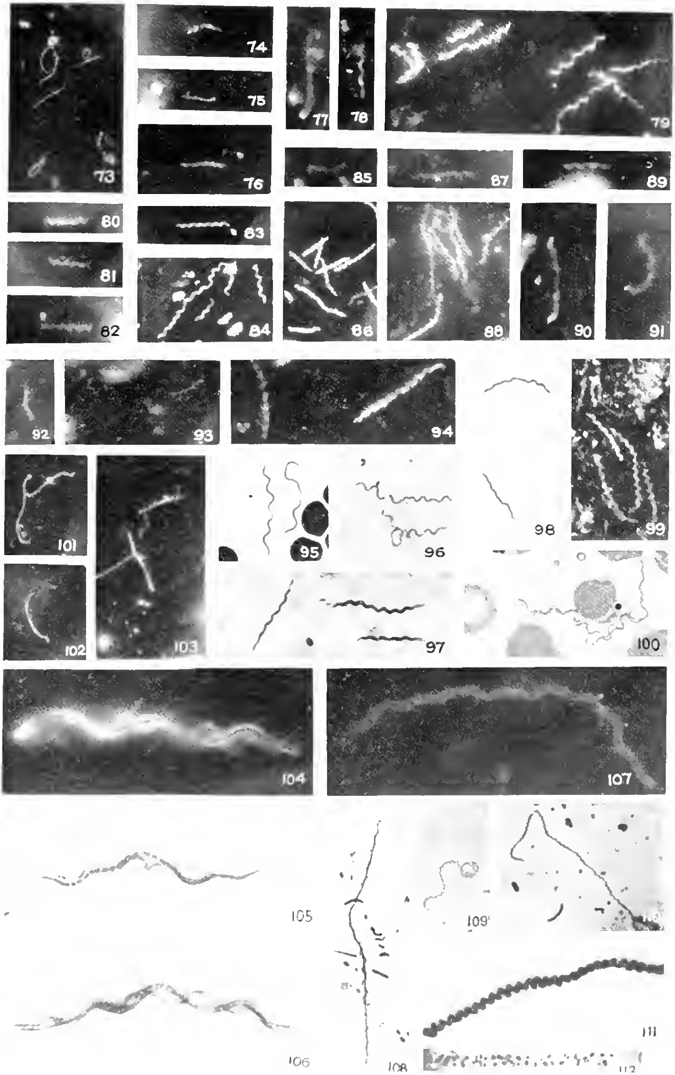


(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



Noguchi: Nomenclature of *Leptospira icterohaemorrhagiae*.



(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)

FURTHER STUDY ON THE CULTURAL CONDITIONS OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ.

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(Received for publication, February 20, 1918.)

The cultivation of *Leptospira* (*Spirochæta*) *icterohæmorrhagiæ*[1] is comparatively simple. It was first accomplished by Inada and his coworkers[2] by means of the method recommended by me for the cultivation of several varieties of blood spirochetes.[3] Later, various techniques for the isolation of this organism on artificial media were proposed by Ito and Matsuzaki,[4] Reiter,[5] Martin, Pettit, and Vaudremer,[6] and myself.[7] While all the methods appear to have given satisfactory results, there is no unanimity as to the best one to be followed in routine work. As far as I am aware, there has been no critical analysis of the conditions requisite for uniform success in obtaining a culture. I wish to report here some of the results of my study of the various strains from Asiatic, European, and American sources.

Necessity of Fresh Serum Constituents for the Growth of Leptospira icterohæmorrhagiæ.

My first cultures of the Japanese, European, and American strains of *Leptospira icterohæmorrhagiæ* were obtained by employing a medium containing about 1 part of normal rabbit serum and 2 parts of Ringer's solution, with the addition of an adequate amount of citrate plasma.[8] The rate of multiplication of the organism is faster at 37°C.

[1] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 575.

[2] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[3] Noguchi, *J. Exp. Med.*, 1912, xvi, 199.

[4] Ito, T., and Matsuzaki, H., *J. Exp. Med.*, 1916, xxiii, 557.

[5] Reiter, H., *Deutsch. med. Woch.*, 1916, xlii, 1282.

[6] Martin, L., Pettit, A., and Vaudremer, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 197.

[7] Noguchi, *J. Exp. Med.*, 1917, xxv, 755.

[8] About 0.5 part.

than at 25°C., but on the whole the first generation grows much more slowly than a later generation. It may be several days before growth is definitely ascertained.

The question may be raised as to what part of the serum is essential for the cultivation of the organism. For the purpose of determining this point, a portion of a mixture of rabbit serum 1 part, and Ringer's solution 3 parts, was heated to 60°C. for 30 minutes and another portion to 100°C. for 15 minutes. Unheated serum was used as control. It was found that heating to 100°C. for 15 minutes destroyed the nutrient value of the rabbit serum. Heating to 60°C. for 30 minutes reduced but did not destroy its cultural value as compared with the control. The nutrient principle of the serum, therefore, is closely associated with coagulable serum proteins. Filtration through the Berkefeld filter does not alter the cultural value of the serum medium.

Comparative Nutrient Value of Various Sera.

Not many animals are susceptible to the inoculation of *Leptospira icterohæmorrhagiæ*, and the guinea pig is the only animal in which the infection is almost invariably fatal. Rabbits are comparatively resistant, 1 to 2 cc. of a well growing pure culture being required to produce jaundice, whereas 0.000001 cc. of the same cultures may cause typical symptoms and death in a guinea pig. Dogs are more sensitive than rabbits, while cats, white rats, mice, and wild rats tolerate the infection and become carriers. A comparison of the suitability of various animal sera for purposes of cultivation of the organism is of practical as well as of biological interest.

Sheep Serum.—Of twelve different sheep sera, only four were found to be suitable, and in these the life of the organism was much shorter than in rabbit serum medium. A mixture of serum 1 part, Ringer's solution 3 parts, and 1.5 per cent agar 0.5 part was used. Undiluted sheep sera gave no better results, nor was the use of the citrate plasma from sheep advantageous.

Guinea Pig Serum.—Eight different lots of guinea pig sera were tested, each lot containing the sera from several animals, and good results were obtained in all. The sera were diluted three times with Ringer's solution and a small amount of agar or citrate plasma was

added. In this medium, however, the organism died out much sooner than in the rabbit serum medium.

Horse Serum.—Two out of four different horse sera proved to be very satisfactory, especially when used in a mixture of 1 part serum, 3 parts Ringer's solution, and 0.5 part 1.5 per cent agar. In this medium the culture survived for many weeks.

Calf Serum.—Only two calf sera were tested, but both gave a fairly good growth. A 1:4 dilution of serum with Ringer's solution was better than the undiluted serum. Martin, Pettit, and Vaudremer[6] recommend a 1:10 dilution of this serum as most suitable.

Goat Serum.—The only serum tested was very suitable when used in a mixture of 1 part serum, 3 parts Ringer's solution, and 0.5 part 1.5 per cent agar. The undiluted serum did not give so good a growth.

Donkey Serum.—The one available specimen proved totally unsuitable.

Pig Serum.—Two pig sera were tested, but the culture failed to grow in any concentration.

Rat Serum.—The sera from about twenty white rats were mixed and tested for their nutrient value. Diluted as well as undiluted sera were employed, but the results were negative.

Human Serum.—Five specimens which had been collected many months previous to the time of testing proved to be without any nutrient value for the organism in question. Two other specimens, which were freshly collected[9] from syphilitic patients, were found to be fairly suitable when used in proportions of 1:1 and 1:3 with Ringer's solution. The culture was short lived, however, reaching its greatest growth in about 11 days at 37°C. and dying off during the following week. The growth of the culture in the rabbit serum control medium was still increasing when the other cultures died.

Ascitic Fluid.—Twenty different samples of ascitic fluid were tested. They were used undiluted and also in different dilutions with Ringer's solution, but up to the present time none has been found suitable for the cultivation of *Leptospira icterohæmorrhagiæ*.

[9] These specimens were obtained through the courtesy of Dr. David J. Kaliski.

Nutrient Value of Organ Emulsions.

In the later stages of infection *Leptospira icterohæmorrhagiæ* invades the visceral organs in enormous numbers, the liver and kidneys being principally involved. One might infer, therefore, that these organs contain an abundant quantity of the substances favorable for the life and multiplication of the organism, and that an emulsion of these organs would constitute an ideal culture medium. The experimental data, however, did not support this assumption.

Emulsions of approximately 5 per cent in Ringer's solution were prepared with the liver, kidney, spleen, heart muscle, and testicle of a normal rabbit and a normal guinea pig, killed by bleeding, and tested for their nutrient value as culture media. In order to make the conditions of the media as varied as possible, the emulsions were used in four different ways: in one set of tubes the emulsion was used alone and unheated, in the second it was heated to 60°C., in the third it was heated to 100°C., and in the fourth there was added agar amounting to 0.3 per cent. The mixture of rabbit serum, Ringer's solution, and citrate plasma and that of rabbit serum, Ringer's solution, and agar were used as control media.[7] In the media containing the organ emulsions no sign of growth of the spirochete was observed, while excellent cultures were obtained in the control media.

The organs of guinea pigs were just as unsuitable for the cultivation of the organism as those of rabbits. I was not unaware of the possible change in the reaction due to autolysis of the organ cells, or of the injurious effect which certain autolytic cleavage products might have, but the emulsions showed a weak alkaline reaction throughout the experiments.

When rabbit serum, in the proportion of approximately 25 per cent, was added to a number of the tubes containing the emulsions, the spirochete multiplied vigorously; therefore, the fact that no culture was obtained with pure organ emulsions must have been due to the absence of suitable nutrient substances for the organism.

Egg White and Egg Yolk as Culture Media.

The failure of various organ emulsions to serve as culture media turned my attention to the possibility of utilizing egg white and egg

yolk for the purpose. The white and yolk of an egg were separated and each was diluted with Ringer's solution in different proportions: 2.5 cc. + Ringer's solution 2.5 cc.; 1 cc. + Ringer's solution 4 cc.; 0.5 cc. + Ringer's solution 4.5 cc.; and 0.25 cc. + Ringer's solution 4.75 cc. In each instance one set of tubes was used in the fresh state and the other heated to 55°C. for 24 hours with a view to possible improvement of nutrient value. In none of the egg media was any culture obtained, nor did the addition of the rabbit serum enhance their nutrient value beyond that of the serum.

Concentration of the Serum in Culture Media.

The importance of the presence of serum for the successful growth of the spirochete having been demonstrated, the following experiments were undertaken in order to determine the influence of various

TABLE I.

Japanese strain.	37°C.		26°C.	
	7 days.	30 days.	7 days.	30 days.
Undiluted rabbit serum..........................	+	+++	+	+++
50 per cent rabbit serum + Ringer's solution....	+	+++	+	+++
33 " " " " + " "	+	+++	+	+++
25 " " " " + " "	+	+++	+	+++
20 " " " " + " "	+	+++	+	+++
15 " " " " + " "	+	+++	+	+++
10 " " " " + " "	+	+++	++	+++
5 " " " " + " "	+	+	+	+++

European strain.	Cultures, 30 days at 26°C.	Proteins precipitable with 10 volumes of absolute alcohol.
33 per cent rabbit serum + Ringer's solution	+++	Copious coarse precipitate and opalescence.
20 " " " " + " "	++	Copious coarse precipitate and opalescence.
10 " " " " + " "	++	Minute granules and opalescence.
5 " " " " + " "	+	Opalescence.
2 " " " " + " "	–	"
1 " " " " + " "	–	Granular.
0.5 " " " " + " "	–	

TABLE I—*Concluded.*

American strains.	Cultures, 30 days at 26°C.		
	Strain 1	Strain 2	Strain 3
33 per cent rabbit serum + Ringer's solution...	+++	+++	+++
20 " " " " + " " ...	+++	+++	+++
10 " " " " + " " ...	++	+++	++
5 " " " " + " " ...	+	++	+
2 " " " " + " " ...	−	−	−
1 " " " " + " " ...	−	−	−
0.5 " " " " + " " ...	−	−	−

The above experiments show that a maximum growth may be obtained with all strains tested in a medium containing more than 20 per cent serum, while a 10 per cent serum medium may give as much growth, but only with certain strains. The growth is scanty in a 5 per cent serum solution, and in a medium containing 2 per cent or less there is no growth.

concentrations of serum upon the culture. Table I summarizes the results.

Influence of Diluents and of Salt Concentration upon the Culture.

The apparent indifference of the spirochete to salt constituents of the culture media was noticed from the beginning of the cultivation

TABLE II.

American strain No. 1	Cultures 30 days at 26° C.
Rabbit serum 1 cc. + 10 per cent sodium chloride 4 cc. = 8 per cent sodium chloride........	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 2 cc. + water 2 cc. = 4 per cent sodium chloride........	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 1 cc. + water 3 cc. = 2 per cent sodium chloride........	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 0.5 cc. + water 3.5 cc. = 1 per cent sodium chloride........	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 0.25 cc. + water 3.75 cc. = 0.5 per cent sodium chloride........	+++
Rabbit serum 1 cc. + water 4 cc. = salt-free control........	+++
" " 1 " + Ringer's solution 4 cc. = serum-Ringer's solution control........	+++

experiments. Instead of Ringer's solution, a 0.9 per cent saline solution or distilled water could be used as a diluent. In fact sewer water and stagnant or ordinary tap water were found to be satisfactory diluents when previously rendered sterile by filtration or autoclaving. The organism displays great tolerance not only to various neutral salts or organic matter which are apt to be present in sewer or stagnant water, but also to an increasing concentration of sodium chloride. The relation of salt concentration to growth is shown in Table II.

There was no perceptible difference in the degree of growth of the organism in this experiment, or in its morphological features. The tonicity of the culture medium is apparently an unimportant factor.

Effect of Reaction upon the Culture.

Leptospira icterohæmorrhagiæ seems to be one of the most sensitive of the microorganisms to the reaction of the culture medium. A slight variation to acid or alkaline from a given optimum zone renders a medium totally unsuitable for the growth of the organism (Table III).

Considering the minuteness of the quantities of hydrochloric acid or sodium hydroxide which were added in these experiments, and the extent to which the reagents were finally diluted with serum and distilled water, one cannot fail to realize the great importance which the reaction of the culture medium must have in relation to the growth of the organism. Similar results were obtained with the Japanese and European strains. The first requisite to the successful cultivation of *Leptospira icterohæmorrhagiæ* appears to be an optimum reaction of the culture medium, which, in my experience, lies between a slight alkaline reaction and that resulting from subsequent multiple dilutions with indifferent diluents (distilled water, isotonic salt solution, Ringer's solution, etc.).

A considerable fluctuation was found by titration of the sera of several domestic animals. For example, 2 cc. of the sera of the sheep, donkey, ox, and pig, each mixed with 3 cc. of distilled water, required 0.4 cc. of 0.1 N hydrochloric acid to bring about a neutral reaction, and 0.6 cc. to cause distinct acidity and turbidity. Rabbit serum had a uniformly weaker reaction, only 0.2 cc. of 0.1 N hydro-

TABLE III.

American strain No. 1	Physical changes.	Reaction to litmus paper.	Result of cultivation at 30° C. for.	
			6 days.	18 days.
Rabbit serum 1 cc. + water 3 cc.	Clear.	Slight alkaline.	+++	++
" " 1 " + 1.5 per cent agar 0.5 cc.	"	Slight alkaline.	+++	++++
Addition of acid.				
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.1 cc.	Slight opalescence.	Neutral.	+	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.1 cc. + 1.5 per cent agar 0.5 cc.	Slight opalescence.	"	+	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.2 cc.	Many sandy precipitates on wall and bottom.	Trace of acid.	–	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.2 cc. + 1.5 per cent agar 0.5 cc.	Slight opalescence.	Trace of acid.	+	–
Addition of alkali.				
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.1 cc.	Clear.	Distinct alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.1 cc. + 1.5 per cent agar 0.5 cc.	"	Distinct alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.2 cc.	"	Stronger alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.2 cc. + 1.5 per cent agar 0.5 cc.	"	Stronger alkaline.	–	–

chloric acid being required to produce a neutral, and 0.35 cc. an acid reaction. The reaction of horse serum lay between that of the rabbit and that of the other animals. The fact that some of the latter sera showed a better nutrient value in dilution may be explained by the reduction of native alkalinity through dilution.

Oxygen Requirement of the Culture.

At the beginning of these cultivation experiments, I supposed *Leptospira icterohæmorrhagiæ* to be an obligatory or facultative anaerobe, because of its great facility for invading organs and multiplying in them. All attempts at cultivation failed as long as cultural conditions were employed which were calculated to produce anaerobiosis. The combination of conditions which I designated as aerotropic anaerobiosis[10] several years ago, and which was successfully used for the cultivation of the relapsing fever spirochetes, gave fairly good results when a suitable serum was used. But in the tubes to which a piece of fresh rabbit kidney was added, the cultures grew less luxuriantly and died out sooner than in the control tubes without the tissue. The simplicity of the cultural requirements of this organism was a surprise and led to the inference that the organism is an aerobe. When a number of subcultures of the Japanese, European, and American strains were cultivated at 37°C. in an anaerobic apparatus and another set without the exclusion of oxygen, excellent growth took place in all tubes where oxygen was accessible, while not a single organism could be found in the tubes kept in an anaerobic apparatus. The tubes were taken out of the anaerobic jar after 12 days and allowed to stand for several days at 37°C., but no new culture developed, probably because of the death of the organism during its stay in the anaerobic apparatus. *Leptospira icterohæmorrhagiæ*, therefore, has been shown to be an obligatory aerobe.

Detrimental Conditions Caused by Physical Hindrances to the Penetration of Oxygen into the Medium.

For obligatory aerobic bacteria a slant or plate agar or broth should be satisfactory, because most of this class of organisms grow in more

[10]Noguchi, *The Harvey Lectures*, 1915–16, 236.

or less discrete, often thick or elevated colonies on the surface of a solid medium. In broth the growth may be diffuse or superficial, forming a pellicle or thick scum. The use of a high layer agar or gelatin for the cultivation of such organisms means a waste of medium, since oxygen cannot penetrate the greater part of it. Since *Leptospira icterohæmorrhagiæ* is an obligatory aerobe, it follows that the addition of solid substances such as agar or gelatin, which must necessarily interfere with the entrance of oxygen into the medium, will be detrimental to the growth of the organism. The denser the concentration of agar or gelatin, the narrower is the zone to which oxygen can penetrate. The experiment summarized in Table IV shows the effect of different concentrations of agar or gelatin upon the culture. The gelatin and agar were made in a 0.5 per cent saline solution and adjusted to a slightly alkaline reaction.

TABLE IV.

Medium.	37°C.			26°C.		
	4 days.	7 days.	28 days.	4 days.	7 days.	28 days.
Gelatin (10 per cent) 4 cc.	–	–	–	–	–	–
Rabbit serum 1 cc. Gelatin (10 per cent) 3 cc.	+	–	–	<<+	–	–
Rabbit serum 1 cc. Gelatin (10 per cent) 1 cc. Ringer's solution 2 cc.	+	+++	+++	<+	+	+++
Rabbit serum 1 cc. Gelatin (10 per cent) 0.5 cc. Ringer's solution 2.5 cc.	+	+++	+++	<+	<+	+++
Agar (2 per cent) 4 cc.	–	–	–	–	–	–
Rabbit serum 1 cc. Agar (2 per cent) 3 cc.	+	++	<+	<<+	+	<<+
Rabbit serum 1 cc. Agar (2 per cent) 1 cc. Ringer's solution 2 "	+	++++	++++	+	++	++++
Rabbit serum 1 cc. Agar (2 per cent) 0.5 cc. Ringer's solution 2.5 "	++	++++	++++	+	++	++++

The experiment demonstrates the disturbing effect of gelatin when present in more than 7.5 per cent and of agar in more than 1.5 per cent. Agar, when added in proportions of 0.5 per cent and 0.25 per cent, considerably improved the cultural conditions. In this concentration it does not perceptibly hinder the penetration of oxygen into the medium and it offers to the spirochetes an ideal semisolid permeable substance. In this respect this particular culture medium is even better than a pure fluid medium. Gelatin, when added in proportions of 2.5 and 1.25 per cent, seems to have been neither beneficial nor detrimental to the growth of the culture.

Ordinary Culture Media and Leptospira icterohæmorrhagiæ.

It would be an economic advantage if a simpler method for the cultivation of this spirochete was devised. No culture was obtained, however, with any of the ordinary media, such as plain and 2 per cent glucose bouillon, Hiss serum water, litmus milk, plain and 2 per cent glucose agar, Loeffler's serum, glycerolated bouillon, and agar. A special bouillon medium formulated by Dr. Kligler was tried—1 per cent peptone, 0.5 per cent sodium phosphate, 0.1 per cent glucose, and 0.5 per cent sodium chloride—but without success. The presence of peptone, broth, casein, glucose, etc., instead of having a nutrient valué for *Leptospira icterohæmorrhagiæ* in a suitable medium such as one containing the necessary amount of rabbit serum, seems to have a definite unfavorable influence upon the culture. The addition of a 10 per cent neutral solution of peptone 4.5 cc., to rabbit serum 1.5 cc., rendered the mixture unsuitable for a culture medium, as is not the case with indifferent diluents such as Ringer's solution, distilled water, or isotonic salt solution. Even the addition of approximately 1.5 per cent peptone suppressed growth to a marked degree. Bouillon or glucose bouillon are not good diluents for making up a culture medium for this organism.

Addition of Carbohydrates to Culture Media.

Akatsu,[11] while working in my laboratory, studied the action of various spirochetes upon many carbohydrates, but he did not find

[11] Akatsu, S., *J. Exp. Med.*, 1917, xxv, 375

definite fermentation phenomena in any of the organisms examined. With *Treponema mucosum* and *Treponema microdentium* a definite increase in the amount of acid was noticed. In the present experiment, the Japanese strain of *Leptospira icterohæmorrhagiæ* was cultivated in two sets of media of fourteen tubes each. In one set the media were made up of 1.5 cc. of rabbit serum, 1 cc. of a 10 per cent solution of carbohydrate, previously sterilized by filtration, 2.5 cc. of Ringer's solution, and 1 cc. of citrate plasma of the rabbit. In the other set 1 cc. of 2 per cent agar (melted) was used instead of the citrate plasma. The fourteen carbohydrates used in both sets were glucose, lactose, maltose, levulose, galactose, saccharose, dextrin, inulin, mannite, dulcite, isodulcite, arabinose, raffinose, and salicin. Tubes without carbohydrate and tubes also which were not inoculated with culture were used as controls.

The new generation of the culture became recognizable within a fortnight at 29°C. by the hazy layer at the top of the columns of culture media. The haze extended downwards from the surface to a depth of 1 to 1.5 cm. By examination under the dark-field microscope, the haze was found to represent dense diffuse colonies of actively multiplying spirochetes. The appearance of the haze was the same in the tubes containing the various carbohydrates as in the sugar-free control tubes. In the set where 1 per cent citrate plasma was used to form loose fibrin, the haze was less distinct but extended as far as 3 or 4 cm. below the surface, and the lower border was not sharply outlined as in the media with semisolid agar. The viability of the spirochetes was as great in the media containing carbohydrates as in those without carbohydrates. The reaction of the cultures failed to indicate any attack by the organism upon the carbohydrates. The reaction remained slightly alkaline to litmus paper as before cultivation, and was entirely comparable with the reaction in the spirochete-free controls.

Special attention was given to the detection of possible morphological modifications in the organisms grown in the presence of the carbohydrates, but none was recognized.

Influence of Temperature upon Cultivation.

Inada and his coworkers[2] found that *Leptospira icterohæmorrhagiæ* grows very well at room temperature, as it does at any temperature up to 37°C., but that at lower temperatures (20–25°C.) the organism survives longer than at 37°C.

I have cultivated three different strains of the spirochete at different temperatures. The results, as recorded in Table V, are self-explanatory. The media used consisted of rabbit serum 1 cc. + Ringer's solution 3 cc. + citrate plasma 1 cc. or 1.5 per cent agar 1 cc.

TABLE V.

Strains.	42°C.		37°C.		30°C.		25°C.		10°C.	
	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.
Japanese.										
Plasma.	–	–	++++	++++	++++	++++	++++	++	++++	++
Agar.	–	–	+++	++++	+++	++++	+	<+	+	<+
European.										
Plasma.	–	–	++	+++	++	++	+	–	+	–
Agar.	–	–	++	+++	++++	++++	+	++	<<+	–
American No. 1.										
Plasma.	–	–	++	+++	++	+++	+	++	+++	++
Agar.	–	–	++	+++	++	++++	++	+++	++	+

The ability of the organism to multiply and remain active a long time at 10°C. is interesting from the epidemiological standpoint. It suggests that certain insects might serve as reservoirs of the virus.

Culture Media Recommended for Leptospira icterohæmorrhagiæ.

As a result of the experiments recorded on the relative nutrient value of various sera, the influence of reaction, oxygen tension, diluents, salts, and various other substances, I have formulated the following media:

A. Rabbit serum	1.5 parts.	
Ringer's solution	4.5 “	
Citrate plasma	1.0 part.	
Paraffin oil to cover the surface.		
B. Rabbit serum	1.5 parts.	
Ringer's solution	4.5 “	
2 per cent agar	1.0 part.	
Paraffin oil to cover the surface.		
C. Rabbit serum	1.5 parts.	Semisolid portion.
Ringer's solution	4.5 “	
2 per cent agar	1.0 part.	
After solidification add:		
Rabbit serum	1.5 parts.	Fluid portion.
Ringer's solution	4.5 “	
Paraffin oil to cover the surface.		

Growth usually begins much sooner in Medium A than in Medium B, but after a month more spirochetes will be found in B. For keeping up subcultures of various strains, Media A and B were simultaneously used in small test-tubes each containing 7 cc. of the composite medium.

For obtaining a large amount of culture, long necked flasks of medium capacity (50 to 100 cc.) were used. It was found best to fill the flasks with the medium to one-half or one-third their capacity and then to cover the surface with a very thin layer of paraffin oil. If the flasks are filled higher than this, oxygen becomes less accessible to the deeper part of the medium, especially when it contains agar. The use of a low layer semifluid medium (B) is based upon the fact, previously mentioned, that unrestricted multiplication of *Leptospira icterohæmorrhagiæ* takes place in such a medium on the surface stratum of 1 to 2 cm. Medium A is similarly semifluid, but the fibrin mass loosens and breaks up in time, especially by repeated withdrawal of the culture with pipettes, rendering the penetration of oxygen almost as easy as in a fluid medium. The flasks containing Medium A may therefore be filled half or two-thirds full, with a thin layer of paraffin oil.

Medium C seems to combine the advantages of Media A and B, the lower stratum being composed of Medium B, upon which, after solidification, is superimposed a mixture of rabbit serum and Ringer's solution (1:3). The medium is then inoculated and covered with a thin layer of paraffin oil. For subcultures, 0.1 or 0.2 cc. of a vig-

orously growing culture is pipetted on the surface of new culture media and then covered with paraffin oil.

D is a medium for acclimated strains. A fluid medium consisting of 1 part of horse or sheep serum and 3 parts of Ringer's solution or salt solution proved to be fairly suitable for strains which had become accustomed to the various media (A, B, C) during a period of several months.

SUMMARY AND CONCLUSIONS.

1. The presence of suitable animal or human serum is essential for the cultivation of *Leptospira icterohæmorrhagiæ.*

2. The nutrient value of serum is considerably reduced by heating to 60°C. for 30 minutes and is destroyed by boiling (100°C). Filtration through a Berkefeld filter does not diminish the nutrient value of the serum.

3. The cultural value of different animal sera varies considerably. It is entirely absent from the sera of the rat and the pig. The sera of the rabbit, horse, and goat are better suited for the growth of the organism than those of the guinea pig, sheep, donkey, or calf. Human serum is suitable, but not ascitic fluid.

4. Fresh or heated emulsions of the liver, kidney, heart muscle, or testicle of the normal guinea pig or rabbit have no cultural value for the organism. The same may be said of both the white and yolk of the hen's egg.

5. A luxuriant growth takes place in a medium of Ringer's solution to which more than 10 per cent of normal rabbit serum is added. There is only moderate growth with 5 per cent of serum, and none when less than 2 per cent is present. The use of an undiluted serum offers no advantage over a diluted one, provided the latter contains at least 10 per cent of serum. In the case of certain animal sera dilution seems to make them more suitable for cultivation purposes, owing perhaps to its reduction of their inherent alkalinity.

6. The tonicity of the culture medium has but little influence upon the growth and morphology of the organism. A medium containing distilled water as diluent or one containing 8 per cent sodium chloride seems to give identical results. The viability of the organism was

greatest in a medium in which Ringer's solution or isotonic salt solution was used as diluent.

7. The reaction of the medium is an important factor in the cultivation of the organism, which thrives most vigorously in a medium of which the reaction is slightly alkaline, not exceeding that of the serum. If the reaction is neutral, the growth is meager, and the culture is short lived. When the reaction of a medium becomes alkaline by the addition of a small amount of sodium hydroxide, or faintly acid by the addition of a little hydrochloric acid, no growth can take place.

8. *Leptospira icterohæmorrhagiæ* is an obligatory aerobe. Any hindrance to the access of oxygen constitutes an unfavorable factor in obtaining a culture.

9. The addition of carbohydrates to media has no perceptible effect upon the growth or morphology of the organism. The reaction of the media is not modified by their presence.

10. *Leptospira icterohæmorrhagiæ* grows at any temperature between 37° and 10°C., the optimum zone being 30–37°C. Growth proceeds more rapidly at 37°C. than at 30° or at 25°, but the cultures remain viable much longer at the latter temperatures. No growth takes place at 42°C.

11. Three different media are described for the cultivation of freshly isolated strains. After prolonged cultivation on these media a strain may be readily cultivated in a serum diluted with Ringer's or isotonic salt solution.

THE SURVIVAL OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ IN NATURE; OBSERVATIONS CONCERNING MICROCHEMICAL REACTIONS AND INTERMEDIARY HOSTS.

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A solution of the question of the survival of *Leptospira icterohæmorrhagiæ* in nature may be brought about (1) by following up directly the actual conditions to which the spirochetes cast off by the hosts or artificially mixed with urine or feces will have to submit, or (2) by mixing the spirochetes with each in turn of the various bacteria commonly encountered in feces, sewage, or soil, and then determining the results of their simultaneous existence in the same media. As I shall show, the spirochete of infectious jaundice is a very delicate organism and is rapidly overwhelmed by most of the bacteria from intestinal contents, sewage, or soil.

Urine in Relation to Leptospira icterohæmorrhagiæ.

The effect of urine upon the viability of *Leptospira icterohæmorrhagiæ* is of practical importance, since it has been found by previous investigators[1, 2, 3] that the urine of about 77 per cent of the patients recovering from infectious jaundice still contains the spirochete after a period of 2 to 5 weeks. They made the interesting observation that two-thirds of the positive urines, some containing numerous spirochetes, failed to produce the infection in guinea pigs. Since the urines came from cases of 15 days' standing or longer, the fact may be ex-

[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1917, xxvi, 341.

[2] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 38.

[3] Cappellani, S., and Frugoni, C., *Sperimentale*, 1917, lxxi, 335.

plained by an attenuation in virulence of the organism during the course of the disease. I have seen one case in which the urine was still infective for guinea pigs 1 month after the onset of the disease.

In one series of experiments a sample of urine freshly collected from a healthy individual, who had no history of ever having had jaundice, was tested for its action upon the spirochetes. 10 cc. amounts of

TABLE I.

Leptospira icterohæmorrhagiæ introduced into.	Changes in appearance and reaction.	0.2 cc. of a well growing culture of American strain No. 1 inoculated into tubes containing.				
		Fluid indicated 6 cc.		Fluid indicated 3.5 cc. + rabbit serum 1.5 cc. + citrate plasma 1 cc.		
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	3 days.
Normal urine	Clear, strongly acid.	–	–	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.1 cc.	Clear, neutral.	<+	–	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0.2 cc.	Precipitate +, slightly alkaline.	<+	–	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0.4 cc.	Precipitate ++, moderately alkaline.	<+	–	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.8 cc.	Precipitate +++, markedly alkaline.	–	–	–	–	–

the urine, which was strongly acid and had a titer such that 10 cc. of it required 7 cc. of 0.1 N sodium hydroxide to become moderately alkaline, were measured into a number of test-tubes, to each of which was added normal sodium hydroxide solution, the quantities added varying in each case in order to obtain a series of reactions from the original acidity of the specimen to a markedly alkaline reaction.

Cultures were set up in two parallel series, using in each series the original and partially neutralized portions of the urine, but adding to one series suitable amounts of rabbit serum and citrate plasma. Table I summarizes the results.

As the table shows, the jaundice spirochetes survived at least 24 hours in the portion of urine to which a quantity of from 0.1 to 0.4 cc. of normal sodium hydroxide solution had been added, but no trace of them could be found in the original urine or in that receiving 0.8 cc. of the alkali. In plain Ringer's solution alone the organism lived 24 hours under similar conditions. After 48 hours there were no spirochetes in any of the tubes of the first series.

The results obtained with the urine containing rabbit serum and citrate plasma were different from those of the other series. There was a good growth in all the tubes containing the unalkalized urine, and also in those to which had been added from 0.1 to 0.4 cc. of normal sodium hydroxide solution. The growth was better and lasted longer in the tubes in which the urine showed a neutral or slightly alkaline reaction than in the unmodified or more strongly alkalized urines. There was no growth in the tube to which 0.8 cc. of the normal sodium hydroxide had been added. While there was unmistakable growth in the urine media with rabbit serum and citrate plasma, the organisms were viable for only 1 week at the longest. The presence of the urine apparently reduces very much the nutrient value of the rabbit serum and citrate plasma, as is shown by the fact that the use of Ringer's solution instead of urine enables the spirochetes to multiply progressively for at least 3 weeks. Not only is the urine devoid of cultural value for the organism, but its presence in an otherwise suitable medium renders the latter less suitable for the growth of the organism.

Feces in Relation to Leptospira icterohæmorrhagiæ.

The escape in feces of living *Leptospira icterohæmorrhagiæ* from experimentally infected guinea pigs seems to be rather frequent. Ido and his coworkers,[1] for example, succeeded in producing typical spirochetosis in seven out of eleven animals tested with a corresponding number of specimens of feces; yet in spite of this high percentage of

TABLE II.

Specimens of feces from which media were prepared.	1:10 dilution.		1:10 dilution, autoclaved.		1:100 dilution, filtrate.	
	No blood.	Blood and serum added.	No blood.	Blood and serum added.	No blood.	Blood and serum added.
Normal feces No. 1	24 hrs.: A few distorted spirochetes among innumerable bacteria. No trace of the spirochetes after 48 hrs. Four daily inoculations (scarified skin) in guinea pigs all negative.	Same as preceding.	A few motile spirochetes after 24 hrs. but none after 48 hrs. No difference between normal and jaundice feces. Four daily inoculations (intraperitoneal) in guinea pigs all negative.	Moderate multiplication of spirochetes in 4 days, which gradually increased. Four daily inoculations (intraperitoneal) all positive.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily inoculations all positive.
" " " 2				Moderate multiplication of spirochetes in 4 days, which gradually increased. No animal inoculations.	Spirochetes survived 4 days, after which they disappeared. No animal inoculations.	A good growth in 4 days which progressed well for 10 days. No animal inoculations.
Jaundice feces No. 1				Moderate multiplication of spirochetes in 4 days, which gradually increased.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily animal

Jaundice feces No. 2				Four daily inoculations. All positive.	One 1st day inoculation positive.	inoculations. All positive.
				Moderate multiplication of spirochetes in 4 days, which gradually increased.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days.
				No animal inoculations.	No animal inoculations.	No animal inoculations.

positive results, it was by no means easy to demonstrate the presence of the spirochetes in feces with the dark-field microscope, the organism being found only once in the stools from 60 guinea pigs having spirochetosis. The specimens of feces which were infective usually contained erythrocytes. In human cases one specimen of feces out of seven was found to be infective.

There is apparently a possibility, then, that the spirochetes are excreted from patients in the feces. To determine the viability of the spirochetes under these conditions, a series of experiments was performed in which they were added in large quantities to feces, both with and without a simultaneous addition of blood ingredients. Two specimens of feces from normal individuals were used, and two from cases of jaundice in children, with the characteristic clay color. Each specimen was used in three ways: (1) as a moderately thick emulsion (1:10) in Ringer's solution, without sterilization; (2) the same sterilized by autoclave; (3) a Berkefeld V filtrate of a dilute fecal emulsion (1:100). Two sets of tubes were set up, each containing 5 cc. of one of the variously prepared suspensions. To each of the tubes of one set were added a few drops of defibrinated blood and 1 cc. of serum from a normal rabbit, and to all the tubes were added 2 cc. of a richly growing culture of either the American or the European strain of the organism. Both sets of tubes were then placed at a temperature of 26°C.

The fate of the spirochetes under these conditions was followed daily by direct microscopic examination and indirectly by inoculation tests on guinea pigs. Table II summarizes the results obtained with the European strain. The results with the American strain were practically identical and are consequently not recorded here.

As is apparent from the table, the spirochete cannot survive in a fecal emulsion, even when there are present sufficient nutrient elements, longer than 24 hours at a temperature of 26°C. That this fact is due to the simultaneous presence of various bacteria, which rapidly overgrow the delicate spirochetes and deprive them of the necessary nutrient substances, is inferred from the much longer survival of the spirochetes in the tubes containing the sterilized emulsion, particularly in those to which were added the blood and serum. In the latter tubes, in fact, there was a temporary multiplication of the

organisms lasting several days. In the tubes containing a dilute, sterile, fecal filtrate, the spirochetes survived at least 4 days, and the addition of blood and serum caused the filtrate to become a suitable culture medium, if not equally as good as Ringer's or saline solution. Inoculations made with the mixtures of the non-sterilized fecal emulsions, with and without the blood and serum and the spirochetes, applied to the scarified skin of guinea pigs, were all negative. Where there was an actual multiplication or survival of the organism, as in the case of sterile suspensions or filtrates, with or without the addition of blood, the animal inoculations were positive.

Judging from the foregoing experiments, it seems highly improbable that, under natural conditions, the causative agent of icterohemorrhagic spirochetosis survives for any length of time after it has left the human body in the feces. It is probably rapidly destroyed by the common bacterial flora of the intestinal tract.

Polluted Water and Soil in Relation to Leptospira icterohæmorrhagiæ.

Samples of water were collected from the East River (a tidal river), from sewage, and from a stagnant cesspool in New York City. It is needless to say that such water is highly contaminated with various bacteria. In one series of experiments the water was used as it was, in another it was autoclaved in order to destroy contaminating bacteria, and in another it was filtered. An emulsion of freshly excreted horse stool was used in one series. The experimental data are given in Table III.

The results show that the spirochetes are not capable of multiplying or even of surviving for any length of time in these contaminated waters. They invariably disappeared in 48 hours. Even when the contaminating bacteria were removed by autoclaving or filtration and rabbit serum was added, only indifferent media resulted, and without the addition of an adequate amount of a suitable nutrient medium (rabbit serum in this experiment) no culture could be obtained.

The question of how long a rich culture of the spirochetes will remain viable when mixed with distilled water and left unprotected from dust in a room was next determined. A Flanders strain, having grown luxuriantly in rabbit serum, Ringer's solution, and agar mix-

TABLE III.

Leptospira icterohæmorrhagiæ introduced into.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.
Ringer's solution 4.5 cc. + rabbit serum 1.5 cc. (control)..	−	+++	Many wks.
East River water 6 cc.	+++	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " " autoclaved, 6 cc.	−	−	
" " " " 4.5 cc. + rabbit serum 1.5 cc.	−	+	2 wks.
Sewer water 6 cc.	+++	−	
" " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " autoclaved, 6 cc.	−	−	
" " " 4.5 cc. + rabbit serum 1.5 cc...	−	+++	Many wks.
Stagnant water 6 cc.	+++	−	
" " 4.5 cc. + rabbit serum 1.5 cc	+++	−	
" " autoclaved, 6 cc.	−	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	−	+++	Many wks.
Horse stool emulsion 6 cc.	+++	−	
" " " 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
" " " autoclaved, 6 cc.	−	−	
" " " " 4.5 cc. + rabbit serum 1.5 cc.	−	+(?)	Accidentally contaminated.
Sewer filtrate 6 cc.	−	−	
" " 4.5 cc. + rabbit serum 1.5 cc.	−	++	More than 3 wks.

ture for 22 days, was placed in distilled water (ten times the volume of the culture) and then allowed to stand in the laboratory without being covered. The distilled water was not sterile, but contained a few large motile bacilli. The results were as follows:

24 hrs: Spirochetes +++; active and long; numerous motile bacilli; fluid slightly opalescent.

48 hrs.:	Spirochetes	+++;	active; more bacilli.
3 days:	"	++;	" " "
4 "	"	+;	" probably more bacilli.
5 "	"	+;	many immobile; " "
6 "	"	<+;	nearly all dead.
7 "	"	−	

The spirochetes remained active and numerous for 48 hours, but all of them gradually disappeared within a week. A drinking water, therefore, richly contaminated with spirochetes, will not be infectious longer than a week.

Samples of soil were collected from several localities in and about New York City for use in an experiment performed to ascertain how long soil will harbor spirochetes under experimental conditions. The samples were rich in organic matter and some came directly from fertilized ground. They were all neutral in reaction. One specimen of soil was obtained from a deeper stratum than the others and was yellowish gray in color. All were purposely contaminated with the spirochetes and determinations of their continued presence in it made daily. No spirochetes could be detected after 72 hours, while there was always an abundance of bacteria. The spirochetes seem to be rapidly overgrown by the contaminating bacteria.

Various Bacteria in Relation to Leptospira icterohæmorrhagiæ.

When the spirochetes are excreted from the infected host, either in the feces or in the urine, their immediate fate will depend upon the presence of various putrefactive bacteria which are always found in the soil in which the feces or urine is deposited. Today we know all of the more common varieties of bacteria that inhabit the intestinal tract or that may be found in unclean objects or soil. There are, of course, a great number of anaerobes as well as aerobes, but since the spirochete in question is an obligatory aerobe,[4] the study of the relation of the bacteria to it becomes much simpler. We have, therefore, to direct our attention only to the part played by aerobic bacteria under natural conditions.

There are many ways of conducting such a study, but I have chosen an indirect one; namely, that of observing the effect of the simultaneous presence of the spirochete in question and each in turn of those bacteria which are likely to coexist with it at the moment when the infected feces, urine, or dead rodent becomes subject to the decomposing forces of the organic world.

A number of culture tubes containing media suitable for the growth

[4] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 593.

of spirochetes was prepared, and all were inoculated with the organism. The tubes were then inoculated with various bacteria and placed in a

TABLE IV.

Bacteria.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.	Remarks as to hemolysis in media.
Control without bacteria	−	+++	Many wks.	−
B. fæcalis alkaligenes	+	+++	12 days.	−
B. aerogenes	++	−	48 hrs.	+
B. cloacæ	++	−	24 “	<+
B. coli	++	−	24 “	<+
B. dysenteriæ Shiga	+	<+	48 “	−
B. “ Flexner-Harris	<+	+	48 “	−
B. typhosus	<+	<+	48 “	−
B. paratyphosus A	++	−	24 “	−
B. “ B	++	−	24 “	−
B. prodigiosus	++	−	24 “	+
B. proteus vulgaris	++	−	24 “	+
B. pyocyaneus	++	−	24 “	+
B. suipestifer	++	−	24 “	−
B. suicidus	++	−	24 “	−
B. subtilis	++	−	24 “	<+
B. mesentericus	+	−	24 “	<+
B. xerosis	+	−	24 “	−
B. sp.? large, motile, chromogenous	+	++	4 days.	−
Streptococcus Pr.	<+	++	5 “	−
“ Brown F 17	++	−	24 hrs.	−
“ “ A 1	±	++	5 days.	−
“ “ C 2	+	++	8 “	−
“ “ W 18	++	<+	3 “	−
“ “ K 4	++	++	5 “	+
“ “ S 6	++	<+	6 “	+
“ “ H 6	+	−	24 hrs.	+
Pneumococcus Type I	++	<+	3 days.	−
“ “ II	++	−	24 hrs.	−
“ “ III	+	<+	3 days.	−
“ “ IV	+	−	24 hrs.	−
Streptococcus aureus	++	+	48 “	<+ slowly.
“ *albus*	++	+	48 “	−

thermostat at the temperature of 26°C. The culture media consisted of 1.5 cc. of rabbit serum, 4.5 cc. of Ringer's solution, 1 cc. of citrate plasma, and 1 drop of defibrinated rabbit blood. Observations were

made of the growth, survival, or disappearance of the spirochetes, the growth of the bacteria, and the presence or absence of hemolysis in the cultures. The results obtained during a period of 2 weeks are recorded in Table IV.

It is apparent from the recorded observations that the more vigorous the growth of a bacterium, the less is the possibility that the spirochetes in the same medium will multiply. The longest period of survival of the spirochetes, except in the control tubes, was observed in the media simultaneously inoculated with *Bacillus fæcalis alkaligenes*. Certain strains of streptococci, notably the non-hemolytic types, seem not to have interfered for a certain period, after which, however, the spirochetes rapidly disappeared from the culture. In the presence of most of the intestinal bacteria, such as *Bacillus coli*, *Bacillus aerogenes*, *Bacillus cloacæ*, etc., the spirochetes were not only unable to multiply but were rapidly destroyed within 24 hours. It may be added that no growth of the spirochetes took place in ordinary bouillon, either with or without the simultaneous inoculation of the bacteria just enumerated. The bacteria grew vigorously in the bouillon.

Microchemical Reactions.

The resistance of various spiral organisms to the solvent action of bile, bile salts, saponin, and sodium oleate has been a subject of study for many years, and it was once thought to differentiate the protozoa from the bacteria. Although this view is no longer valid, because some bacteria have been found to act like protozoa and *vice versa*, the fact is of sufficient interest to make worth while a determination of the resistance of the present organism to these reagents (Table V).

The jaundice spirochetes appear to be highly sensitive to the destructive action of the bile[5] and bile salts when employed in concentrations of 1:30 or more, while saponin exhibited no injurious effect upon them, even when used in as high a concentration as 10 per cent. The action of sodium oleate was stronger than that of the bile or bile salts and produced a granular disintegration of the organism in a dilution of 1:10,000. Among the organisms which under-

[5] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 41.

TABLE V.

Reagent.	Results in different concentrations.									
	1:10			1:30		1:100			1:300	1:1,000
	After 5 min.	After 30 min.	After 5 hrs.	After 5 min.	After 5 hrs.	After 5 min.	After 30 min.	After 5 hrs.	After 5 hrs.	After 5 hrs.
Ox bile.	Still active.	None motile, nearly all shadow forms.			All shadow forms.			Some affected. Nearly all active.	No effect. All active.	
Rabbit bile.	Many inactive.	All shadow forms.			All shadow forms.			All shadow forms.	Some affected. Majority active.	
Sodium taurocholate.	All shadow forms.	Shadow forms.	Shadow forms less distinct.	All immobile. Better preserved in form.		Nearly all active.	All shadow forms.		Nearly all active.	No effect. All active.
Sodium glycocholate.	Nearly all shadow forms.		All shadow forms.		All shadow forms.			Some still active.	Nearly all active.	No effect. All active
Sodium oleate.	All dead; distorted and granular.			All dead; distorted and granular.				Nearly all gone; few motile.	Nearly all gone; few motile.	Nearly all gone; few motile, but more active than those in the 1:300 dilution.
Saponin.			No effect. All active.		No effect. All active.			No effect. All active.	No effect. All active.	

went this disintegration, however, was a number of actively motile, apparently intact organisms.

The destructive action of the rabbit bile as well as of the bile salts and sodium oleate was considerably reduced by the addition of serum, as shown in Table VI.

Ido and his coworkers[1] observed that in spite of the difficulty of finding spirochetes in the bile when it was examined under the dark-field microscope, two out of three specimens of the bile of guinea pigs dying of experimental spirochætosis icterohæmorrhagica were capable of producing typical infection in the guinea pig. This

TABLE VI.

Leptospira icterohæmorrhagiæ introduced into.	10 per cent rabbit bile 1 cc. + culture 1 cc.	10 per cent sodium taurocholate 1 cc. + culture 1 cc.	10 per cent sodium oleate 1 cc. + culture 1 cc.	0 + culture 1 cc.
Rabbit serum 0.5 cc.	No apparent effect. All active.	Nearly all active.	Nearly all active.	All motile.
60 per cent rabbit serum 0.5 cc.	Many gone, some motile.	Many dead and distorted. A few motile.	Many active.	" "
20 per cent rabbit serum 0.5 cc.	Nearly all gone.	Nearly all gone.	" "	" "
6 per cent rabbit serum 0.5 cc.	All gone.	All gone.	Nearly all gone.	" "
2 per cent rabbit serum 0.5 cc.	" "	" "	All gone.	" "
Ringer's solution 0.5 cc.	" "	" "	" "	" "

may be ascribed to the fact that in these specimens of bile there was mixed a certain amount of the blood and also the serous exudate from the affected liver, which, by virtue of their well known inhibitory effect upon the solvent action of the bile salts, must have protected some spirochetes from destruction in the bile. Guinea pig bile was affected by the serum in the same way.

A parallel series of experiments with a specimen of ox bile obtained from an abattoir gave somewhat contradictory results. In this instance the addition of the rabbit or horse serum failed to check the destruction of the organism by this bile, which had a much stronger

solvent power than that of the rabbit or guinea pig. At all events, the amount of the serum necessary to nullify the destructive action of the bile is so large that the escape of the spirochetes in the bile seems less probable than would appear from the observations of the investigators just quoted. Perhaps the impairment of hepatic function through the spirochetal infection of the organ may lead to a decrease of the bile salts in such a specimen.

Leptospira icterohæmorrhagiæ and Intermediary Hosts.

It has been shown by previous investigators that the spirochetes may remain in the organs of certain rodents without producing serious illness, and that they may be excreted in the urine. From the experiments already described, it seems improbable that the spirochete can survive very long after leaving the infected hosts. The infection of man, therefore, must result from contact with the spirochete before its destruction under natural conditions; that is, the carrier rodents must be present in places frequented by man. But while this source of infection may explain many cases of infection, there are a few in which the infective agent cannot be traced in this way.

The question of insect carriers has been taken up by Reiter,[6] who obtained only negative results with certain biting flies, fleas, and bedbugs. In the present study opportunities were afforded the writer to ascertain whether or not the larvæ of certain varieties of flies or mosquitoes could become infected with spirochetes when fed on infected guinea pig liver or raised in a stagnant water tank into which an abundance of the culture had been put.

The larvæ of the common house-fly were allowed to feed for 2 days on infected material consisting of several pieces of the liver and kidney of a guinea pig killed in the last stage of experimental Weil's disease. They were then transferred to a clean receptacle and fed for 5 days on a non-infected mass of horse manure, and at the end of that time they were crushed into an emulsion and smeared over depilated areas of the skin of guinea pigs. The emulsion was also examined for spirochetes under the dark-field microscope. The examination revealed no spirochetes, and the guinea pig remained normal.

[6] Reiter, H., *Deutsch. med. Woch.*, 1916, xlii, 1282.

A similar experiment with the larvæ of bluebottle flies (*Calliphora vomitoria*) gave only negative results.

In another series of experiments, about 50 cc. of a rich culture of spirochetes (Japanese strain) were added to 150 cc. of stagnant water in which twenty-five mosquito larvæ had been living for some time. The water was neutral in reaction and was quite clear and transparent at the time when the culture was introduced. The larvæ swam about actively in the usual manner after the addition of the culture. A drop of the contaminated water examined under the dark-field microscope contained numerous active spirochetes. There were a few bacteria. After 24 hours at room temperature, the water became somewhat turbid. Most of the larvæ were still active, but the number of the spirochetes was diminished and that of the bacteria increased. At the end of 48 hours there was a scum of bacteria over the surface of the water and no spirochetes could be found. All but six of the largest larvæ had died. The water was full of bacteria and infusoria. It is possible that the death of the mosquito larvæ and of the spirochetes was the result of overcrowding by the bacteria and infusoria, increased suddenly by the addition of the culture media to the water. The surviving larvæ were kept in the same water for 5 days and then crushed into an emulsion to be used for an infection experiment on a guinea pig and also for examination under the dark-field microscope. The results were entirely negative.

Another series of experiments was performed with adult mosquitoes (*Culex pipiens*) by first allowing them to feed on an infected guinea pig, in the blood of which had been found spirochetes, and then, after 6 days, causing them to bite normal guinea pigs. No infection resulted from their bites.

Wood ticks (*Dermacentor andersoni*) failed in several experiments to transmit the infection from guinea pig to guinea pig. Leeches (*Hirudo medicinalis*) were allowed to suck blood from an infected guinea pig until their bodies were engorged. In the blood escaping from the wound inflicted by the leeches a few spirochetes could be found under the dark-field microscope. These "infected" leeches were kept at room temperature for 7 days and afterwards in a cool room at 15°C., being taken out at the end of intervals of 2, 3, 4, 6, and 8 months and made to suck normal guinea pigs, but so far no infection

has been produced. Some of the leeches died in the meantime, but those which still survived at the end of the 3 month interval were examined for the presence of spirochetes. The viscid, dark reddish, decomposed (?) blood showed no spirochetes under the dark-field microscope, nor did it cause infection when tested on guinea pigs. Some of the tissues were examined by the silver impregnation method, but with negative results. Apparently there is no multiplication of the spirochetes after their ingestion by leeches, and no infection can be induced by the bite of the latter after a period of 1 week.

SUMMARY AND CONCLUSIONS.

1. *Leptospira icterohæmorrhagiæ* is unable to grow in the urine, either with or without the addition of suitable culture ingredients, the acidity of the urine being detrimental to the growth. It survives less than 24 hours, unless the urine is neutralized or slightly alkalized, when the period of survival is somewhat longer. If suitable nutrient ingredients are added to the neutralized or slightly alkalized urine, the organism is able to grow for about 10 days, after which multiplication ceases.

2. Feces from normal or jaundiced persons destroy *Leptospira icterohæmorrhagiæ* within 24 hours when a rich culture is added and the mixture allowed to stand at 26°C. The addition of blood serum and corpuscles does not prevent the destruction of the organism. Autoclaved specimens and filtrates of unheated feces do not constitute a suitable medium in which to keep the organism alive for any length of time, but the addition of blood corpuscles and serum in adequate quantities renders them fairly satisfactory as media. Under natural conditions *Leptospira icterohæmorrhagiæ* cast off in the feces cannot survive more than 24 hours.

3. Polluted water, sewage, and soil will not serve to keep *Leptospira icterohæmorrhagiæ* alive for more than 3 days at the most. When deprived by filtration or autoclaving of their bacteria they become indifferent diluents and may be used to make up a culture medium when mixed with serum and citrate plasma of a suitable animal. Sterilized soil with a neutral reaction, when added to a culture, has an unfavorable effect upon the growth of the organism.

4. Most of the aerobic bacteria found in feces, sewage, soil, and tap water inhibit the growth of *Leptospira icterohæmorrhagiæ* when inoculated into the same medium. *Bacillus fæcalis alkaligenes* and many strains of non-hemolytic streptococci caused the least interference, although growth was never so vigorous or lasting in the media in which they were present as in the control media. Certain pathogenic bacteria (*Bacillus typhosus*, *Bacillus paratyphosus*, *Bacillus dysenteriæ*, pneumococcus) are antagonistic to the growth of the spirochete.

5. *Leptospira icterohæmorrhagiæ* is highly sensitive to the destructive action of bile, bile salts, and sodium oleate, but resists the action of saponin. In this last respect it differs from many so called spirochetes. The destructive action of these agents is counteracted by blood serum.

6. The larvæ and adults of the *Culex* mosquito, the larvæ of the house-fly and bluebottle fly, wood ticks (*Dermacentor andersoni*), and leeches failed to become carriers of the spirochetes when fed on infected guinea pigs or their organs; that is, they cannot play the part of an intermediary host of *Leptospira icterohæmorrhagiæ*.

BEHAVIOR OF HYPOCHLORITE AND OF CHLORAMINE-T SOLUTIONS IN CONTACT WITH NECROTIC AND NORMAL TISSUES IN VIVO.

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It has been known for some time that the chlorine content and consequently the potency of hypochlorite of soda solutions diminishes rapidly when in contact with the surface of wounds. This is emphasized by Carrel and Dehelly, and for this reason they advocate a frequent renewal of the antiseptic solution to the wound.[1] This insures that the concentration shall be kept as constant as possible.

It would be difficult to determine the rapidity of the fall in chlorine concentration on an actual wound as encountered in the ward, and almost impossible to parallel such observations with others on an equal quantity of solution in contact with an equal area of normal skin. Inasmuch as exact determinations of the rapidity of the fall in chlorine concentration on pathological and on normal skin, under experimental conditions, might be of value to surgeons using Dakin's hypochlorite and chloramine-T solutions clinically, we chose the following method of investigation.

The left ears of three white rabbits of the same relative size and weight were exposed to the rays emitted by a Coolidge tube. The spark-gap used measured 3 inches; the milliamperage was 10; the distance from the target to the ear was 6 inches; and the time of exposure was 20 minutes.

8 weeks later the x-rayed ears each exhibited a sharply demarcated gangrenous area over which there were considerable crusting of epithelium and secretions and in the lumen there was much thick pus.

The ears of the affected rabbits were each suspended for 20 minutes in a beaker containing 400 cc. of the solution to be tested.

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 65.

Rabbit 1.—Right and left ears suspended in separate beakers containing Dakin's hypochlorite solution, made from bleaching powder, 10 cc. of which required 13 cc. of 0.1 N sodium thiosulfate solution for reduction (0.48 per cent sodium hypochlorite).

Rabbit 2.—Right and left ears suspended separately in beakers containing a solution comparable in alkalinity with properly made Dakin's hypochlorite solution—sodium carbonate 1 gm. and sodium bicarbonate 17 gm. per liter of water.

Rabbit 3.—Right and left ears suspended separately in chloramine-T[2] solution which required 12.75 cc. of sodium thiosulfate for reduction (about 2 per cent chloramine-T), and contained approximately the same proportion of available chlorine as the hypochlorite solution used on Rabbit 1.

TABLE I.

Solution.	In contact with.	Before.	Immediately after.	2 hrs. after.	17 hrs. after.
		cc.	*cc.*	*cc.*	*cc.*
Dakin's hypochlorite solution.........	Normal ear.	13.00	12.35	12.15	11.50
" " "	Gangrenous ear.	13.00	11.55	10.30	8.65
" " "	Control (no tissue).	13.00	13.00	13.05	12.60
Carbonate-bicarbonate "	Normal ear.				
" "	Gangrenous ear.				
Chloramine-T solution...............	Normal ear.	12.75	12.75		12.75
" "	Gangrenous ear.	12.75	12.75		12.35
" "	Control (no tissue).	12.75	12.75		12.75

In the tables the figures represent the number of cubic centimeters of 0.1 N sodium thiosulfate solution required to reduce the chlorine in 10 cc. of the solution.

Table I shows the titration figures before, immediately after the 20 minutes' exposure of the normal and necrotic ears to the solutions, 2 hours after the ears had been removed from the solutions, and 17 hours after removal. The solutions were kept in covered vessels at room temperature in the interval between titrations.

The fall in chlorine concentration was more rapid in the Dakin's hypochlorite solution applied to the gangrenous ear than in that applied to the normal ear. The fall in concentration, however, was not complete immediately after the ears were removed from the solution but became more pronounced the longer the interval between

[2] Prepared by the Abbott Laboratories, Chicago.

the removal of the ears and the titration. The titration of the control solution, which had not been exposed to any tissue, demonstrated a fall in the titration figure from 13 to 12.60 cc., and a small proportion of the loss in chlorine of the solutions in contact with the tissues might be explained by this spontaneous deterioration of the unstable hypochlorite solutions. However, this factor is insufficient to account for the fall from 13 cc. before exposure to 8.65 cc. 17 hours later in the gangrenous ear, nor from the same figure before to 11.50 cc. at the end of the 17 hour interval in the normal ear. The fall from 13 cc. before exposure to 11.55 cc. immediately afterward is associated with the erosive action of the hypochlorite solutions, which we have measured quantitatively in a former investigation,[3] but the cause of the further fall to 8.65 cc. is not immediately clear. The fluid in contact with the gangrenous ear was cloudy immediately after the removal of the ear. This cloudiness of the fluid was not so marked at the end of the 2 hour interval when the second titration was made, and the fluid was almost as clear as the control at the end of the 17 hour interval when the last titration gave the lowest chlorine concentration recorded. Close inspection of the fluid immediately after the removal of the ear revealed the presence of small particles of necrotic tissue, flecks of pus, etc., in suspension. These became less noticeable the longer the antiseptic solution was allowed to act. The fall in chlorine concentration exhibited immediately after the removal of the ears was due to the erosive effect of the solution on the necrotic tissue, and to its combination with the products of the tissues *in situ*. However, during this action, appreciable particles of necrotic tissue, agglomerations of pus cells, and little gummy concretions made up of dried serum, epithelial cells, etc., were separated from the necrotic ear and it is the subsequent reaction of the hypochlorite with these which caused the continued fall in the chlorine titer. That the chlorine is directly concerned in this solvent action seems assured from former experiments reported.[3] In the course of the reaction the chlorine probably goes into such stable union with the protein substances that it is not available to the sodium thiosul-

[3] Taylor, H. D., and Austin, J. H., The solvent action of antiseptics on necrotic tissue, *J. Exp. Med.*, 1918, xxvii, 155.

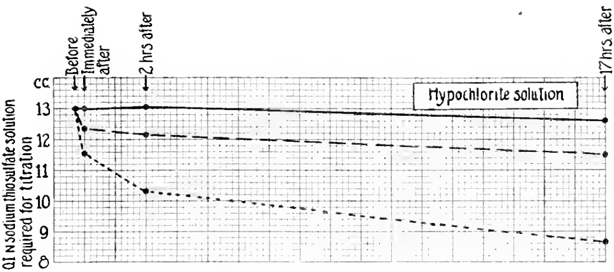
fate solution, and thus the fall in titer measures the exact quantity of chlorine used up in the reaction with the wound products.

The fall in chlorine concentration of the hypochlorite solution in contact with the normal ear, much less pronounced than with the x-rayed ear, was probably due to the erosive action of the solution on the hair and superficial epithelium of the normal ear and the slow digestion of the particles removed caused the slow fall noted over the interval of 17 hours. As there was much less tissue capable of reacting with the solution in the normal than in the gangrenous ear, the titration figures were higher at all observations in the solution exposed to the former. Close observation revealed the erosive effect on the ear itself.

The fall in chlorine concentration noted in the chloramine-T solutions was much less than that observed in the hypochlorite solutions. This corresponds with the greater stability of the former and with their lack of erosive effect on necrotic tissue.[3] It is interesting to note that there was no fall in chlorine concentration in the chloramine-T solution applied to the normal ear, and correspondingly no erosive action on the hair or superficial epithelium was demonstrable. The solution was likewise clear when removed from the ear and throughout the period of observation, in contrast to the hypochlorite solution which was at first cloudy and only late in the experiment became relatively clear. The titration figures after 17 hours were approximately the same as those made immediately after the removal of the ears from the solution. Text-fig. 1 shows graphically the fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue. Text-fig. 2 gives comparable curves for the chloramine-T solutions.

A weaker hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), was applied to the gangrenous ear and to the normal ear of Rabbit 1, and titration figures before, immediately after removal of the ears from the solution, 2 hours afterward, and 17 hours afterward were compared with a control solution which was never in contact with tissue. These results, shown in Table II and Text-fig. 3, confirm those shown in Table I and Text-fig. 1 for Rabbit 1.

The ears of each rabbit were then suspended in solutions of the same types and concentrations as those shown in Table I for 7 consecutive days, the period of exposure on each day being 20 minutes. At the end of this time it was seen that the gangrenous ear suspended in Dakin's hypochlorite solution had cleared up proportionately more



——— Control: no tissue.
- - - - - - - Normal ear.
. . . . X-rayed ear.

TEXT-FIG. 1. The fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

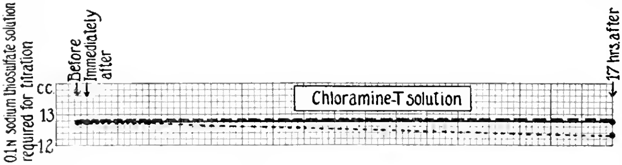
TABLE II.

Solution.	In contact with.	Before.	Immediately after.	17 hrs. after.
		cc.	*cc.*	*cc.*
Dakin's hypochlorite solution	Normal ear.	9.0	8.5	8.25
" " "	Gangrenous ear.	9.0	7.0	5.5
" " "	Control (no tissue).	9.0	9.0	8.9

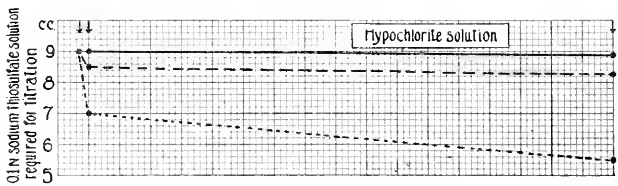
than the gangrenous ears of either of the other rabbits. The scabs were more eroded and the pus present was less in amount than in either of the others. The gangrenous area looked much cleaner. The chloramine-T gangrenous ear, which was not so severe an x-ray burn in the beginning, had not improved appreciably. The gangrenous ear

treated with the control alkaline solution had not changed in appearance.

The normal ears of the chloramine-T and of the alkaline control rabbits were just as they had been before treatment with these substances. The normal ear treated with the hypochlorite solution was



TEXT-FIG. 2.



TEXT-FIG. 3.

TEXT-FIG. 2. The fall in chlorine concentration in the chloramine-T solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

TEXT-FIG. 3. The fall in chlorine concentration of the second hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), applied to the gangrenous ear, in that applied to the normal ear, and in the control which was not allowed to act on any tissue.

intensely inflamed. It was twice as thick as it had been before it was treated, due to intense edema. Congestion was marked and the surface temperature was higher than normal. There was superficial ulceration in places and petechiæ were scattered through the subcutaneous tissues.

CONCLUSIONS.

1. The fall in chlorine concentration of Dakin's hypochlorite solution is more rapid in contact with necrotic than in contact with normal tissue.

2. The fall in chlorine concentration of chloramine-T solution is very slight when applied to necrotic tissue and is negligible when applied to normal tissue.

3. The action of the hypochlorite solution on tissue results in the separation of particles of necrotic tissue, hair, epithelial scales, coagulated serum, etc., and a gradual digestion of these substances, taking place over a period of at least 17 hours.

4. The fall in the chlorine concentration of the hypochlorite solution is not complete until the particles are completely dissolved.

5. Chloramine-T solution, 2 per cent, has no erosive effect comparable with that exhibited by the hypochlorite solution.

6. Repeated exposures to the three solutions show the hypochlorite solution to be superior in its cleansing ability on necrotic tissue.

7. The hypochlorite solution is much more irritating to normal rabbit skin than chloramine-T solution or the alkaline control solution.

8. Therefore, the irritating effects must be due to the readily available chlorine.

TOXICITY OF CERTAIN WIDELY USED ANTISEPTICS.

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In view of the widespread use of certain antiseptics in the treatment of infected wounds, it has seemed desirable to make toxicity tests on animals under conditions in which rapid absorption might be expected. While, as a rule, the antiseptics are employed under conditions that preclude the possibility of much absorption with consequent systemic effect, yet occasionally certain of them have been recommended and in some instances even used for injection into closed cavities. This practice, which would probably lead to considerable absorption, has not been general, the majority of surgeons proceeding with much caution.

A few experiments of Carrel and Dehelly[1] demonstrated that Dakin's hypochlorite solution when injected subcutaneously in the guinea pig was relatively non-toxic, one-fortieth of the body weight of the animal injected being borne without demonstrable ill effect. Bashford[2] has tested the toxic effect of dilute hypochlorite solutions on the living tadpole immersed in Dakin's solution. Inasmuch as these experiments were few and no data for comparing the relative toxicity of a series of antiseptics were given, it was decided to investigate the toxic action of a number of antiseptic substances in common use. The method was to inject increasing doses into mice intraperitoneally and into guinea pigs both subcutaneously and intraperitoneally, and to note and tabulate the results.

Method.

White mice of approximately 20 gm. and guinea pigs of 300 to 600 gm. body weight were employed and the amount of chemical used is

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 32.

[2] Bashford, E. F., *Lancet*, 1917, ii, 595.

based on the weight of each animal. In regard to the chlorinated antiseptics, the lethal dose is calculated in terms of the sodium hypochlorite equivalent and the available chlorine of the antiseptic. The percentage of the total weight of the antiseptic substance represented by the sodium hypochlorite equivalent is given in a foot-note to Table I.

In Table I the results of the intraperitoneal injection in mice of increasing doses of the antiseptics studied are tabulated. In Column A of Table IV will be found a condensed summary of these results, the antiseptics being arranged in the order of their decreasing toxicity for the animals. Control injections of four of the vehicles employed, namely water, isotonic saline solution, sterile paraffin oil, and Dakin and Dunham's bland oil solvent for dichloramine-T (chlorcosane[3]), show these to be well borne in larger doses than those employed in any of the injections with antiseptics into the test animals. Eucalyptol, however, which has been used in combination with paraffin oil as a vehicle for dichloramine-T is so toxic that its use in experiments of the nature of those recorded here is impossible. The diluting vehicle has been water or isotonic saline solution with all antiseptics except dichloramine-T.

In Table I are given in detail the results of the experiments in which mice were used. The nature and strength of the solution injected, the amount of the solution in cubic centimeters, the amount of the drug in actual milligrams administered and in milligrams per 100 gm. of body weight, with the final results, are recorded.

Table II gives in the same way the results obtained with the few guinea pigs that were injected subcutaneously.

In Table III are recorded the results of the experiments in which guinea pigs were injected intraperitoneally. The form of the table is the same as in Tables I and II.

Table IV summarizes the results given in Tables I to III, showing the greatest dose per 100 gm. of body weight that the animals were able to survive and the smallest dose necessary to kill with the antiseptics named.

[3] The chlorcosane was kindly given us by Dr. H. D. Dakin and Dr. E. K. Dunham. Dakin, H. D., and Dunham, E. K., *Brit. Med. J.*, 1918, i, 51.

DISCUSSION.

It will be seen from the tables that the only antiseptic of which the smallest fatal dose was smaller than the largest survival dose was dichloramine-T. Since two mice survived 4.7 mg. per 100 gm. of body weight, it is probable that 15.5 mg. rather than 1.6 mg. is to be considered the smallest fatal dose for this series. The distribution of the drug in the viscid bland oil used as a vehicle is probably uneven, which may account for the somewhat variable results obtained with this antiseptic both in mice and in guinea pigs.

Of all the substances tested, eucalyptol and brilliant green are the most toxic, the lethal dose of each being 0.1 mg. per 100 gm. of body weight. Mercurophen,[4] mercuric chloride, and chloramine-T constitute the group with the next highest toxicity, the lethal dose being 1 mg. per 100 gm. of body weight. Dichloramine-T, proflavine,[5] and the four hypochlorite solutions tested follow in the order named with a lethal dose of about 10 to 15 mg. per 100 gm. of body weight. The least toxic chemicals are iodine and phenol, of which the lethal doses are about 50 mg. per 100 gm. of body weight.

In Table II are recorded a few experiments with the antiseptics injected under the skin of the abdomen of guinea pigs. The lethal dose of Dakin's hypochlorite solution per 100 gm. of body weight is the same as that determined intraperitoneally in the mouse. Chloramine-T and dichloramine-T administered in this manner gave rise to local necrosis with extensive sloughing. It is probable that only a small part of the drug injected reached the general system of the animal and in consequence the determination of the lethal dose in this way can hardly be considered satisfactory. It was accordingly abandoned and five of the antiseptics were tested in guinea pigs by intraperitoneal injections. The results are tabulated in Table III and summarized in Column B of Table IV.

Chloramine-T has the same toxicity per unit of body weight for guinea pigs and for mice. The same may be true of dichloramine-T or this substance may be somewhat less toxic for the guinea pig.

[4] The mercurophen was sent to us for trial through the kindness of Dr. J. F. Schamberg.

[5] The proflavine was obtained from England.

TABLE I.

Results of Injecting Mice Intraperitoneally.

Animal No.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
		cc.		*mg.*	*mg.*	
1	Sodium chloride 0.85 per cent	0.50	Sodium chloride.	4 20	20.00	Lived.
2	" " 0 85 " "	1 00	" "	8.50	40.00	"
3	" " 0.85 " "	4 00	" "	34.00	170 00	"
4	Distilled water	0.50				Lived.
5	" "	2.00				"
6	" "	4 00				"
7	Dakin's hypochlorite 0 41 per cent (bleaching powder)	0 10	Sodium hypochlorite.	0 40	2 00	Lived.
8	" " 0 41 " " " "	0.50	" "	2.00	10.00	"
9	" " 0.47 " " (original)	0.50	" "	2.30	12.00	"
10	" " 0.49 " " (bleaching powder)	0.50	" "	2.40	12.00	"
11	" " 0.50 " " (neutral)*	0.50	" "	2.50	12.00	"
12	" " 0.47 " " (original)	1.00	" "	4.70	24.00	Died in 15 hrs.†
13	" " 0.50 " " (neutral)*	1.00	" "	5.00	25.00	" " 18 " †
14	" " 0.41 " " (bleaching powder)	2.00	" "	8.20	40.00	" " 3 "
15	" " 0.47 " " (original)	2.00	" "	9.40	47.00	" " 15 " †
16	" " 0.41 " " (bleaching powder)	4.00	" "	16.40	80.00	" " 1½ "
17	Hychlorite, 0.5 per cent sodium hypochlorite	0.33	Sodium hypochlorite.	1.70	8.00	Lived.
18	" 0.5 " " " "	0.33	" "	1.70	8.00	"
19	" 0.5 " " " "	0.50	" "	2.50	12.00	"
20	" 0.5 " " " "	0.50	" "	2.50	12.00	Died in 6 hrs.

21	Chloramine-T 0.2 per cent	0.10	Sodium hypochlorite.	0.05	0.26	Lived.
22	" 0.2 " "	0.30	" "	0.16	0.80	"
23	" 2.0 " "	0.10	" "	0.50	2.60	Died in 19½ hrs.
24	" 2.0 " "	0.50	" "	2.60	13.00	" " 2 "
25	" 2.0 " "	0.50	" "	2.60	13.00	" " 1½ "
26	" 2.0 " "	0.50	" "	2.60	13.00	" " 2½ "
27	Dichloramine-T 0.5 per cent in bland oil	0.10	Sodium hypochlorite.	0.30	1.60	Lived.
28	" 0.5 " " " " "	0.10	" "	0.30	1.60	Died in 30 hrs.
29	" 0.5 " " " " "	0.30	" "	0.90	4.70	Lived.
30	" 0.5 " " " " "	0.30	" "	0.90	4.70	"
31	" 5.0 " " " " "	0.10	" "	3.10	15.50	Died in 18 hrs.†
32	" 5.0 " " " " "	0.10	" "	3.10	15.50	" " 18 "†
33	" 5.0 " " " " "	0.20	" "	6.20	31.00	" " 18 "†
34	" 5.0 " " " " "	0.20	" "	6.20	31.00	" " 18 "†
35	" 5.0 " " " " "	0.33	" "	10.60	50.00	" " 2 "
36	" 5.0 " " " " "	0.50	" "	15.50	75.00	" " 1½ "
37	" 5.0 " " " " "	0.50	" "	15.50	75.00	" " 1½ "
38	Paraffin oil	0.50				Lived.
39	Bland oil	0.33				"
40	" "	0.50				"
41	Mercuric chloride 0.01 per cent	0.10	Mercuric chloride.	0.01	0.05	Lived.
42	" " 0.01 " "	0.30	" "	0.03	0.15	"
43	" " 0.10 " "	0.10	" "	0.10	0.50	"
44	" " 0.10 " "	0.50	" "	0.50	2.50	Died in 18 hrs.
45	Mercurophen 0.01 per cent	0.50	Mercurophen.	0.05	0.25	Lived.
46	" 0.10 " "	0.10	"	0.10	0.50	"
47	" 0.10 " "	0.30	"	0.30	1.50	Died in 48 hrs.
48	" 0.10 " "	1.00	"	1.00	5.00	" " 27 "

* Cullen, G. E., and Austin, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 41.
† Died over night.

TABLE I—*Continued.*

Animal No.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
		cc.		*mg.*	*mg.*	
49	Brilliant green 0.01 per cent	0.10	Brilliant green.	0.01	0.05	Lived.
50	" " 0.01 " "	0.30	" "	0.03	0.15	Died in 48 hrs.
51	" " 0.10 " "	0.10	" "	0.10	0.50	" " 6 "
52	" " 0.10 " "	0.50	" "	0.50	2.50	" " 18 "
53	" " 0.10 " "	0.50	" "	0.50	2.50	" " 3½ "
54	Proflavine 0.1 per cent	0.33	Proflavine.	0.33	1.70	Lived.
55	" 1.0 " "	0.10	"	1.00	5.00	"
56	" 1.0 " "	0.50	"	5.00	25.00	Died in 18 hrs.†
57	" 1.0 " "	0.50	"	5.00	25.00	" " 12 "
58	" 1.0 " "	1.00	"	10.00	50.00	" " 3 "
59	" 1.0 " "	1.00	"	10.00	50.00	" " 18 "
60	Tincture of iodine 7.0 per cent	0.10	Iodine.	7.00	35.00	Lived.
61	" " " 7.0 " "	0.30	"	21.00	100.00	Died in 45 min.
62	" " " 7.0 " "	0.50	"	35.00	175.00	" " 10 "
63	" " " 7.0 " "	1.00	"	70.00	350.00	" " 15 "
64	Commercial hypochlorite (Javelle water) 0.5 per cent of sodium hypochlorite	0.50	Sodium hypochlorite.	2.50	12.00	Lived.
65	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite	0.50	" "	5.00	25.00	Died in 4½ hrs.
66	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite	1.00	" "	10.00	50.00	" " 2½ "

67	Peroxide of hydrogen 3.0 per cent (commercial)	0.50	Peroxide of hydrogen.	15.00	75.00	Lived.
68	Magnesium hypochlorite equivalent to 0.5 per cent sodium hypochlorite	0.50	Sodium hypochlorite.	2.50	12.00	Lived.
69	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	0.50	" "	5.00	25.00	Died in 25 min.
70	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	1.00	" "	10 00	50.00	" " 10 "
71	Phenol 0.25 per cent	0.50	Phenol.	1.25	6.20	Lived.
72	" 1.00 " "	0 30	"	3.00	15.00	"
73	" 1 00 " "	0 50	"	5.00	25 00	"
74	" 1.00 " "	1 00	"	10 00	50 00	"
75	" 1 00 " "	1 00	"	10 00	50 00	Died in 5 min.
76	" 1 00 " "	1.50	"	15 00	75 00	" " 12 "
77	Eucalyptol 10 0 per cent in paraffin oil	0.10	Eucalyptol.	0.01	0 05	Lived.
78	" 10.0 " " " " "	0.30	"	0.03	0 15	Died in 4 hrs.
79	" 100 0 " "	0.10	"	0 10	0 50	" " 10 min.
80	" 50.0 " " in paraffin oil	0.50	"	0 25	1.25	" " 10 "

Sodium hypochlorite equivalent of

Dakin's hypochlorite	= 100	per cent.
Commercial hypochlorite (Javelle water)	= 100	" "
Hychlorite	= 100	" "
Magnesium hypochlorite	= 117.4	" "
Chloramine-T	= 26.5	" "
Dichloramine-T	= 62.1	" "

TABLE II.

Results of Injecting Guinea Pigs Subcutaneously.

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug	Amount of drug per 100 gm. of body weight.	Result.
	gm.		*cc.*		*mg.*	*mg.*	
1	400	Dakin's hypochlorite 0.5 per cent	10.0	Sodium hypochlorite.	50.00	12.00	Lived.
2	550	" " 0.5 " "	13.7	" "	68.00	12.00	"
3	425	" " 0.48 " "	10.6	" "	51.00	12.00	"
4	475	" " 0.5 " "	11.9	" "	60.00	13.00	"
5	450	" " 0.48 " "	22.5	" "	108.00	24.00	Died in 12 hrs.
6	350	Hychlorite, 0.5 per cent sodium hypochlorite	9.0	Sodium hypochlorite.	45.00	13.00	Lived.
7	450	Chloramine-T 2.0 per cent	11.25	Sodium hypochlorite.	59.00	13.00	Lived (sloughed).
8	550	" 2.0 " "	13.7	" "	73.00	13.00	" "
9	450	Eucalyptol 50.0 per cent	11.25	Eucalyptol.	5,600.00	1,200.00	Died in 12 hrs.
10	350	Dichloramine-T 5.0 per cent in bland oil	9.0	Sodium hypochlorite.	280.00	83.00	Lived (sloughed).
11	575	Proflavine 0.1 per cent	7.2	Proflavine.	7.20	1.25	Lived.
12	300	" 0.1 " "	7.5	"	7.50	2.50	"

TABLE III.

Results of Injecting Guinea Pigs Intraperitoneally.

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
	gm.		*cc.*		*mg.*	*mg.*	
13	507	Dakin's hypochlorite 0.5 per cent	3.10	Sodium hypochlorite.	15.00	3.00	Lived.
14	350	" " 0.5 " "	4.37	" "	22.00	6.30	Died in 9½ hrs.
15	375	" " 0.5 " "	9.37	" "	47.00	12.50	" " 7½ "
16	566	Hychlorite, 0.5 per cent sodium hypochlorite.	3.50	Sodium hypochlorite.	17.00	3.00	Lived.
17	500	" 0.5 " " " "	6.25	" "	31.00	6.20	Died in 3¼ hrs.
18	450	" 0.5 " " " "	11.25	" "	56.00	12.50	" " 7½ "
19	430	Chloramine-T 0.2 per cent	2.70	Sodium hypochlorite.	1.40	0.30	Lived.
20	600	" 2.0 " "	1.20	" "	6.40	1.10	"
21	502	" 2.0 " "	3.10	" "	16.00	3.20	Died in 3 hrs.
22	425	" 2.0 " "	5.30	" "	29.00	6.70	" " 1½ "
23	450	" 2.0 " "	11.25	" "	59.00	13.30	" " 1 hr.
24	525	Proflavine 0.1 per cent	6.55	Proflavine.	7.00	1.30	Lived.
25	525	" 0.1 " "	13.10	"	14.00	2.60	"
26	597	" 0.1 " "	30.00	"	30.00	5.00	Died in 72 hrs.
27	470	Dichloramine-T 0.5 per cent in bland oil.	1.90	Sodium hypochlorite.	5.90	1.30	Lived.
28	470	" 0.5 " " " " "	5.90	" "	18.30	3.90	Died in 22 hrs.
29	550	" 5.0 " " " " "	2.20	" "	68.00	12.30	Lived.
30	498	" 5.0 " " " " "	6.20	" "	193.00	39.00	Died in 1½ hrs.
31	375	" 5.0 " " " " "	4.70	" "	146.00	39.00	" " 2 "
32	375	" 5.0 " " " " "	9.37	" "	292.00	78.00	" " 1 hr.

TABLE IV.

Summary.

Drug.	A. Mice, injected intraperitoneally.		B. Guinea pigs, injected intraperitoneally.		C. Guinea pigs, injected subcutaneously.	
	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Eucalyptol	0.15	0.05				
Brilliant green	0.15	0.05				
Mercurophen	1.50	0.50				
Mercuric chloride	2.50	0.50				
Chloramine-T	2.60	0.80	3.2	1.1		
Dichloramine-T	1.60 (?)		3.9 (?)			
	15.50	4.70	39.0	12.3		
Proflavine	25.00	5.00	5.0	2.6		
Hychlorite	12.00	12.00	6.2	3.0		
Dakin's hypochlorite	24.00	12.00	6.3	3.0	24.0	13.0
Commercial hypochlorite (Javelle water)	25.00	12.00				
Magnesium hypochlorite	25.00	12.00				
Iodine	100.00	35.00				
Phenol	50.00	50.00				

All the figures represent milligrams of antiseptic, or in the case of the chlorinated antiseptics, milligrams of sodium hypochlorite equivalent of the antiseptic, per 100 gm. of body weight.

Proflavine, hychlorite,[6] and Dakin's hypochlorite solution given intraperitoneally are all about two or three times as toxic per 100 gm. of body weight for the guinea pig as for the mouse. On the whole, however, the toxicity of the antiseptics follows about the same order in the two species of animal. When the great difference in the body weight of the mouse and the guinea pig is considered, the constancy of the lethal dose per unit of body weight is striking.

While it is, of course, not justifiable to calculate arbitrarily, on the basis of body weight alone, the fatal dose of these substances for man, it is interesting in this connection to note that if such a compu-

[6] Made by General Laboratories, Madison, Wisconsin.

tation could be considered valid the following amounts of certain of the antiseptics under the proper circumstances would constitute a fatal dose for a man weighing 70 kg.

0.14 cc. of equal parts of paraffin oil and eucalyptol (formerly considerably used as a solvent for dichloramine-T).

144 cc. of a 2 per cent solution of chloramine-T.

160 cc. of a 5 per cent solution of dichloramine-T in bland oil.

1,600 cc. of any of the hypochlorite solutions tested, having sodium hypochlorite titration of 0.5 per cent.

However, only a small amount of the antiseptic employed is absorbed from wound surfaces or from an abscess cavity, and little if any danger from constitutional effects would be expected from their employment in this way. When used in closed cavities, in the serous cavities of the body, or when sutured within a wound, these figures should, we believe, be kept in mind. This is especially the case in respect to eucalyptol used as a vehicle.

The drugs are tabulated in Table IV in the order of diminishing toxicity. It is interesting to note that the least toxic drugs that are efficiently bactericidal are the hypochlorite series and iodine.[7, 8, 9] The only two of the four hypochlorite solutions studied that are suitable for clinical use are Dakin's hypochlorite solution (in this case made from bleaching powder) and hychlorite. Of the other substances which vary somewhat in their greater toxicity for mice and guinea pigs, the most efficient are proflavine, dichloramine-T, chloramine-T, and possibly brilliant green. Mercurophen, mercuric chloride, and phenol can be disregarded as having too feeble disinfecting powers. Eucalyptol, the most toxic substance included in this study, is not recommended as a bactericidal agent, but merely as a solvent for dichloramine-T. Of these drugs, the only ones having appreciable solvent action on necrotic tissues, pus, etc., are the hypochlo-

[7] Dakin, H. D., and Dunham, E. K., Handbook of antiseptics, New York, 2nd edition, 1918.

[8] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

[9] Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

rites.[10, 11] Chloramine-T and the hypochlorites have also a destructive action on bacterial toxins.[12]

CONCLUSIONS.

1. The substances injected intraperitoneally into mice and guinea pigs arranged in the order of their decreasing toxicity are: eucalyptol and brilliant green; mercurophen; mercuric chloride and chloramine-T; dichloramine-T and proflavine; hychlorite, Dakin's hypochlorite, Javelle water, and magnesium hypochlorite; iodine and phenol.

2. Now that Dakin's bland solvent, chlorcosane, is available as a vehicle for dichloramine-T, eucalyptol should probably be discarded for this purpose because of its much greater toxicity.

3. Inasmuch as experienced surgeons do not approve of the injection of solutions of iodine and phenol into closed cavities, it would seem advisable not to use any of the antiseptics here discussed in this manner inasmuch as all exhibit a greater toxicity for mice and guinea pigs than the two chemicals first named.

4. The method of testing toxicity of antiseptics by subcutaneous injection is not satisfactory because exudation and subsequent sloughing reduce the rate of absorption and make uncertain the amount finally absorbed.

[10] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

[11] Austin, J. H., and Taylor, H. D., *J. Exp. Med.*, 1918, xxvii, 627.

[12] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 375.

THE EFFECT OF PAINTING THE PANCREAS WITH ADRENALIN UPON HYPERGLYCEMIA AND GLYCOSURIA.

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INTRODUCTION.

About a year after the discovery by Blum[1] that subcutaneous injections of adrenal extracts produce glycosuria, Herter and his coworkers[2] published several communications on the sugar-producing effect of adrenal substance when administered intraperitoneally. They employed adrenalin chloride (Takamine, 1: 1,000). According to Herter, intraperitoneal injections of adrenalin give better results than subcutaneous or intravenous injections. Herter's communications contain further the following striking statements: that painting of the pancreas with adrenalin brings on glycosuria which amounts often to 10 per cent and more, and that such a remarkable production of glycosuria may be brought about even by painting only one-fifth of the pancreas with adrenalin. He further states that "the pronounced nature of the glycosuria following intraperitoneal injections appears to be mainly attributable to the readiness with which the adrenalin comes into contact with the pancreas." The glycosuric action of painting the pancreas is brought into connection with the fact that glycosuria invariably makes its appearance after complete depancreatization, and the theory is advanced that the painting of the pancreas with adrenalin acts upon the cells of this organ in the manner of the action of hydrocyanic acid; namely, by the prevention of oxygenation of the glandular cells. In the discussion following this communication at the meeting of the Association of American Physicians, Herter stated that he was unable to offer a satisfactory reply to the question of one of us; namely, why the prevention of oxygenation of the cells of one-fifth of the pancreas should produce glycosuria, while the removal of even more than one-half of that gland produces no glycosuria. To the remarks of Cushing that the operative procedure,

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[1] Blum, F., *Deutsch. Arch. klin. Med.*, 1901, lxxi, 146.

[2] Herter, C. A., and Richards, A. N., *Med. News*, 1902, lxxx, 201. Herter, C. A., *ibid.*, 1902, lxxx, 865. Herter, C. A., and Wakeman, A. J., *Tr. Assn. Am. Phys.*, 1902, xvii, 570; *Virchows Arch. path. Anat.*, 1902, clxix, 479. Vosburgh, C. H., and Richards, A. N., *Am. J. Physiol.*, 1903, ix, 35.

etc., might have been responsible for the glycosuria, Herter said that he "used only ether, and after you have used this for hours on dogs and examined the urine you get only a trace of sugar or none whatever." He admits that in a few instances the amount of glycosuria is inconsiderable and in exceptional instances glycosuria may be entirely absent. However, Herter's papers do not contain figures recording the number of successful experiments after painting the pancreas or after intraperitoneal injections, or indicating the degrees of the results.

In Herter and Wakeman's communications[2] there is only an unimportant reference to the hyperglycemic effect of painting. But later Vosburgh and Richards[2] report eight experiments in which the hyperglycemia was studied, five after painting the pancreas and three after intraperitoneal injections of adrenalin chloride. In these instances the increase in hyperglycemia was, indeed, considerable; but no statement is made as to the glycosuric effects. Vosburgh and Richards acknowledge the influence of ether upon the sugar content of the blood and the necessity of having controls for the experiments; they consider as a control the first analysis for blood sugar which has been made after anesthesia has been established and before adrenalin is used. In two of these controls the glycemia was high (0.239 and 0.258 per cent).

These statements are striking. Although more than 14 years have passed the experiments seem not to have been confirmed by other workers. We report below the results which were obtained in the repetition of some of the experiments.

EXPERIMENTAL.

Method.—The experiments were made on dogs. They were anesthetized with ether, some by the intratracheal insufflation method, some received ether by cone or towel, and in others ether was insufflated through a cone. In all cases urine and blood were analyzed for sugar content, the blood was analyzed once before painting the pancreas with adrenalin (Parke, Davis and Company, 1: 1,000), and several times at intervals after painting. Blood was obtained from a cannula in an artery (carotid or femoral) or from the external jugular vein by means of a syringe, and the urine from a catheter kept permanently in the bladder. Probably because of the etherization, the amount of urine obtained was usually small. The experiment was observed for several hours; that is, at least until the glycemia was distinctly on its descending course. The urine was examined at intervals after painting and in some instances the urine excreted during the following 18 or 20 hours was collected and examined. Etheriza-

tion was continued either throughout the entire experiment or until the painting and with it the operative handling of the animal was finished. The quantitative analysis for sugar was made by Myers and Bailey's modification of the Lewis-Benedict method.[3]

After laparotomy the pancreas was exposed to as full a view as possible. At least one-third of the organ was painted. In some experiments the entire quantity of the adrenalin was painted on at once; in others about the same quantity was used but divided for painting several times. The results of the two methods are presented in Tables I and II.

Since the adrenalin will reach the pancreatic cells more thoroughly if applied after the thin membrane and the external covering of connective tissue has been removed, this was done in most of the experiments.

The results will be found in compact but full detail in the tables. Table I presents those obtained in seven experiments in which the pancreas was painted only once with fairly large quantities.

As regards the blood sugar we are concerned essentially with the determinations made during etherization just before exposure of the pancreas and the highest percentage of the sugar found at any time after painting it. The difference between these two determinations indicates the effect of painting the pancreas with adrenalin. Taking the highest increase during an experiment as characteristic of the effect of the painting, the rise in the blood sugar content in the experiments of Table I is as follows: 0.09, 0.07, 0.05, 0.06, 0.00, 0.08, and 0.06 per cent. It varies between 0.09 and 0.00 per cent. The urine never contained reducing substances before painting the pancreas with adrenalin. The highest percentages of glycosuria after painting in the experiments of Table I are as follows: 1.27, 5.00, 0.00, 0.93, 0.68, 0.94, and trace. As can be seen from the data in the table, the percentages of sugar in the urine following the painting were irregular, the highest being 5 per cent and the lowest 0.00 per cent. Evidence of definite relation between glycosuria, hyperglycemia, and the amount of adrenalin used in painting the pancreas is lacking.

Table II summarizes five experiments in which the pancreas was painted several times. The entire quantity of adrenalin used in

[3] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.

TABLE I.

Application of Adrenalin to the Pancreas Once.

Experiment No.	Weight. Sex.	Part and proportion of entire pancreas painted.	Amount of adrenalin (1:1,000 solution).	Blood sugar. Before painting. Before ether.	Blood sugar. Before painting. During ether.	Blood sugar. After painting. Time.	Blood sugar. After painting. Percentage.	Urine. Length of period.	Urine. Amount.	Urine. Sugar.	Albuminuria.	Anesthesia.	Temperature of dog.	Remarks
	kg.		cc.	per cent	per cent	hrs. min.	per cent	hrs. min.	cc.	per cent			°C.	
1	16.4 Male.	About one-third (posterior end and body). About one-half of both sides stripped of connective tissue covering.	2.75	0.11	0.12	14	0.15				None before.	Ether (intratracheal insufflation) until 53 min. before last blood sample.	38.2	
						44	0 13							
						→ 1 44	0 21							
						2 45	0 15		14.5	1.27	+		39.1	
													38.4	
2	9 25 Female.	Entire pancreas. Not stripped of connective tissue covering.	3.0	0.15	0.29	5	0.33				None before.	Ether (intratracheal insufflation) throughout.		Died during night. Duodenum, cecum, and colon intensely hemorrhagic.
						15	0.31							
						31	0.33						37.5	
						1 1	0.33	1 3	10.5	4.65			36.6	
						2	0.34							
						3	0.36	2 1	15	5.00	+		35.9	
3	17.5 Male.	Entire pancreas (posterior end on only one side). Stripped only in one small area.	3.1	0.12	0.16	5	0.21				+ before.	Ether (intratracheal insufflation) throughout.		Died during night. Pneumonia.
						16	0.18							
						33	0.18							
						1 1	0.18	1	3.2	0.00	++		36.8	
						2 1	0.20						35.9	
						3 2	0.21	2	2.4	0.00			36.6	

4	12.25	About two-thirds (posterior end and middle). Stripped only in two small areas.	2.4	0.12	0.23		6	0.27					None before.	Ether until 2 hrs. after painting (intratracheal insufflation).	37.	
							16	0.29								
							32	0.28								
	Male.					1	1	0.28	1		About 3	0.0	++		35.0	
						→ 2		0.28							36.7	
						3		0.27	2		11	0.93	++		37.9	
						24		0.13	21		>138	0.0	0		39.1	
													None before.	Ether by cone for 44 min.	38.5 before.	
5	14.5	About one-third (posterior end and middle). Partly stripped.	2.0	0.13	0.21	→	9	0.21		16	1.25	0 0	+		38.3	
						2	24	0.14	2	5	15.5	0.68	+		38.9	
	Male.					4	31	0.12	2		22	0.00	+		38.5	
						22		0.12	20		>188	0.0	Tr.		38.8	
6	7.25	About two-thirds (anterior end and middle). Not stripped.	2.0	0.12	0.14	→	10	0.22		19	A few drops.	0.0	+ before.	Ether blown through cone for about 50 min.	38.3	Died during night. Left lung somewhat congested.
	Male.					2	19	0.20	2	11	>10	0.94	+			
						4	45	0.11	1	50	> 3	0.0	+			
									Post mortem.		21	0.0	+			
													+ before.	Ether blown through cone for about 39 min.		
7	9.6	One-third by weight (middle). Not stripped.	1.0	0.13	0.16	→	12	0.22		20	4	0.0	+			Vomited while under ether and again afterwards.
						2	54	0.14	2	49	12	Tr.	+		39.1	
	Male.					22	30	0.14	19		>38	0.0	Tr.		39.3	

In the tables the arrow indicates the time when ether administration was discontinued.

TABLE II.

Application of Adrenalin to the Pancreas Several Times.

Experiment No.	Weight. Sex.	Part and proportion of entire pancreas painted.	Amount of adrenalin (1:1,000 solution).	Blood sugar. Before painting. Before ether.	Blood sugar. Before painting. During ether.	Blood sugar. After painting. Time.	Blood sugar. After painting. Percentage.	Urine. Length of period.	Urine. Amount.	Urine. Sugar.	Albuminuria.	Anesthesia.	Temperature of dog.	Remarks.
	kg.		*cc.*	*per cent*	*per cent*	*hrs. min.*	*per cent*	*hrs. min.*	*cc.*	*per cent*			*°C.*	
											None be-fore.			
8	11.35	One-half (posterior	2 0	0.14	0.28	→ 10	0.30	11	4	4.44	Tr.	Ether by towel	39.0	Vomited while
		end and part of				2 25	0.21	1 54	13	6.95	"	for 41 min.	38.5	coming out of
	Male.	middle). Ex-				5 20	0.13	2 54	20	3.55	"			ether. Pan-
		treme tip				23	0.13	17		Tr.(?).				creas painted
		stripped.												twice on one
														side and three
														times on the
														other side.
											Bloody			
9	11.05	One-fourth (poste-	1.5	0.15	0.24	→ 10	0.29	15	13.5	4.36	before.	Ether blown	38.8	Each side of pan-
		rior free end							{ 8	2.70	Bloody.	through	37.7	creas painted
	Female.	only). One side				2 53	0.14	2 35	{ 20.5	3 69	Tr.	cone for 57	38.8	twice.
		stripped.				4 34	0.13	1 31	26	Tr.	+	min.	39.5	
						22 36	0.12	18		0			40.1	

													Bloody before.			
10	10	One-third (poste-	1.5	0.12	0.14	→	10	0.17		15	2	0	Bloody.	Ether blown	39.7	Each side of pan-
		rior free end).				2	33	0.12	2	13	15	Tr.	+	through cone	40.3	creas painted
	Female.	Stripped to very				4	32		3	4	25	0	+	for 34 min.	40.3	three times.
		small extent.				24	25	0.13	18	50	240	0	+		39.5	
11	11.1	One-third of	1.5	0.13	0.24	→	11	0.33		15	2.6	3.08	Tr. before.	Ether blown	38.8	Each side of pan-
		length (poste-				4	20	0.12	3	46	23	1.81	++	through	38.2	creas painted
	Male.	rior free end).				23		0.12	19	30	?	0	+	cone for 49	38.0	three or four
		One side												min.		times.
		stripped.														
													Tr. before.		39.0	
12	11.1	One-sixth of length	1.5	0.12	0.18	→	10	0.24		16	3.6	3.57	++	Ether blown	38.0	Each side of pan-
		or one-fourth of			12 min.	2	40	0.13	2	20	20	3.07	++	through	39.0	creas painted
		weight (part of			later	4	35	0.11	1	55	25	0.00	++	cone for 50	39.2	four times.
	Male.	middle and very			0.20.	22	28	0.14	18		?	0.00	++++	min.	38.5	
		little of poste-														
		rior free end).														
		Both sides														
		stripped.														

the several paintings in each of the five experiments in Table II was generally smaller than those used in the experiments of a single painting in Table I. The rise of the blood sugar in the experiments of Table II is as follows: 0.02, 0.05, 0.03, 0.09, and 0.04 per cent. The highest percentages of sugar in the urine in these experiments were: 6.95, 4.36, trace, 3.08, and 3.57. The increase of the blood sugar is about the same in both tables. The glycosuria generally ran higher in the second series of experiments.

A consideration of both series shows that our experiments bear out in a general manner the statement made by Herter, that in the majority of the cases (nine out of twelve of our animals) painting the pancreas with adrenalin is followed by glycosuria, and further, the amount of the glycosuria varies in different experiments. However, our results do not support unqualifiedly the results presented by Herter and the views expressed by him. As regards glycosuria produced by adrenalin, Herter states that subcutaneous administration produces the least amount, intraperitoneal the next highest amount, and painting the pancreas the highest amount. The impression conveyed is that painting the pancreas will, as a rule, produce high glycosuria, 10 per cent and higher. Thus in one experiment 14 per cent was found, while the slighter glycosuria produced was exceptional and rarely the conditions failed entirely. Now our experiments give rather the opposite impression. Thus in one instance only did the glycosuria approach 7 per cent, while in another it was about 5 per cent. On the other hand, of the twelve experiments, three gave no glycosuria or a mere trace. In seven experiments the glycosuria was small or at least not high. The average of the twelve experiments was 2.23 per cent. In other words, we found the glycosuria following painting of the pancreas with adrenalin a less striking phenomenon and we are not convinced that it differs from the similar condition which follows the subcutaneous or intraperitoneal injection of the drug. Two more points must be considered. First, we employed larger amounts of adrenalin than apparently did Herter and his coworkers. Second, in most experiments we removed the connective tissue and the thin membrane from the part of the pancreas which was to be painted with adrenalin, a device which according to Herter[4] is "apt to be most effective."

[4] Herter, C. A., and Wakeman, A. J., *Tr. Assn. Am. Phys.*, 1902, xvii, 577.

The experiments we have made would seem not to support the chief contention of Herter that the "pronounced nature of the glycosuria following intraperitoneal injections appears to be mainly attributable to the adrenalin which comes into contact with the pancreas."

Although Vosburgh and Richards[2] adopt the view of Herter that the glycosuria produced by injection of adrenalin into the peritoneal cavity is of pancreatic origin, they do not mention the point in the conclusion. As already stated, these authors studied only the effects of adrenalin upon glycemia. They made only three experiments, using large, toxic doses of the adrenalin solutions intraperitoneally; one animal died in 24 hours. We wish now to compare the hyperglycemia obtained in our experiments by painting the pancreas with the results obtained by Vosburgh and Richards. Their Table I contains five such experiments of which the main facts are reproduced in our Table III.

The blood sugar rises in their experiments are: 0.102, 0.118, 0.280, 0.240. and 0.052 per cent. Except in one experiment (No. 7) the rise in the blood sugar is therefore considerably higher than that obtained in our twelve experiments. The average increase in our experiments is 0.054 per cent; the average increase in those of Vosburgh and Richards is 0.158 per cent; thus the average of our experiments is about one-third that of Vosburgh and Richards. The doses of adrenalin employed in four of their experiments were larger than those in ours. Analysis shows, however, that the quantity of adrenalin employed by them did not exert an unmistakable effect upon the blood sugar. Thus in Experiment 4, in which 4 cc. of adrenalin were used, the increase amounted only to 0.118 per cent, while in Experiment 6, in which only 2 cc. were used, the rise was 0.240 per cent. Other points of difference between the methods employed by Vosburgh and Richards and by ourselves exist. The amount of blood withdrawn for each analysis is one of these points. Vosburgh and Richards undoubtedly have withdrawn much larger quantities of blood than we have withdrawn in our experiments. Possibly this is a contributing factor in their finding higher blood sugar than we found.[5] The other possibility of a deeper anesthesia and some de-

[5] For example, see Rinderspacher, K., *Biochem. Z.*, 1910, xxvii, 67–72.

TABLE III.

Blood Sugar Content in Five Experiments on Painting the Pancreas with Adrenalin from Table I of Vosburgh and Richards (Abbreviated).

Experiment No.	Time when blood was withdrawn.		Sugar.	Remarks.
			per cent	
3	3.00 p.m.	Normal	0.112	Etherization continued throughout experiment; 3 cc. of adrenalin chloride solution (1:1,000) applied to pancreas with brush at 3.08 p.m.
	3.15 "	7 min. after painting	0.182	
	3.23 "	15 " " "	0.178	
	3.38 "	30 " " "	0.188	
	4.08 "	1 hr. " "	0.204	
	5.06 "	2 hrs. " "	0.214	
	6.00 "	3 " " "	0.165	
4	7.55 p.m.	Normal	0.173	Etherized from 7.45 p.m. till end of experiment; 4 cc. of adrenalin chloride solution (1:1,000) painted on surface of pancreas at 8.12 p.m.
	8.17 "	5 min. after painting		
	8.27 "	15 " " "	0.277	
	8.42 "	30 " " "	0.237	
	9.12 "	1 hr. " "	0.291	
	10.12 "	2 hrs. " "	0.256	
5	10.45 a.m.	Normal	0.239	Ether given throughout experiment; 3 cc. of adrenalin chloride (1:1,000) applied at 10.55 a.m.
	11.00 "	5 min. after painting	0.291	
	11.10 "	15 " " "	0.354	
	11.25 "	30 " " "	0.388	
	11.54 "	1 hr. " "	0.433	
	12.54 p.m.	2 hrs. " "	0.477	
	1.54 "	3 " " "	0.519	
	2.55 "	4 " " "	0.465	
6	4.52 p.m.	Normal	0.131	Ether given throughout experiment; 2 cc. of adrenalin chloride solution (1:1,000) applied to pancreas at 5 p.m.
	5.05 "	5 min. after painting	0.205	
	5.15 "	15 " " "	0.217	
	5.30 "	30 " " "	0.264	
	6.00 "	1 hr. " "	0.315	
	7.00 "	2 hrs. " "	0.371	
7	3.34 p.m.	Normal	0.154	Ether given throughout experiment; 3 cc. of adrenalin solution applied to pancreas at 3.43 p.m.
	3.49 "	6 min. after painting	0.192	
	4.01 "	18 " " "	0.173	
	4.22 "	39 " " "	0.191	
	4.58 "	1 hr., 15 min. after painting	0.206	
	5.43 p.m.	2 hrs. after painting	0.143	
	6.37 "	3 " " "	0.169	

gree of asphyxia (factors which tend to increase the amount of blood sugar) is eliminated by the statement of Vosburgh and Richards that "care was taken to keep the anesthesia as light and as constant as possible." The differences in the method of analysis employed by Vosburgh and Richards (precipitation by phosphotungstic acid and determination of sugar by the Allihn method), and by ourselves (Lewis-Benedict method) cannot be responsible for the variations in our results.

However that may be, we are justified in pointing out the fact that the production of hyperglycemia by painting the pancreas with adrenalin in our experiments was not of unusual degree. There is reason to doubt that the glycosuria and the increase in the hyperglycemia thus produced are greater than would have been produced by the application of adrenalin to any other part of the peritoneal cavity. We have not established by direct experiments the degree of hyperglycemia and glycosuria which is produced by painting some other surface of the peritoneal cavity which would have entitled us to a direct comparison between painting the two surfaces.

On the other hand, we have ascertained definitely that the hyperglycemia and glycosuria produced by intraperitoneal injection of adrenalin is not of pancreatic origin. We have isolated in two series of experiments a part of the pancreas in such a way (1) that the adrenalin applied to the pancreas could not find its way to other parts of the peritoneal cavity at all or to any extent, and (2) that the adrenalin injected into the peritoneal cavity could not reach the isolated part of the pancreas. Table IV records the experiments in which the isolated pancreas was painted with adrenalin.

In the experiments in which the adrenalin was painted on the pancreas and was prevented from coming in contact with any other part of the peritoneum the rise of blood sugar was: 0.02, 0.04, 0.02, 0.06, 0.02, 0.01, 0.04, and 0.07 per cent, giving an average of 0.035 per cent, which is about two-thirds that obtained when the adrenalin was applied to a pancreas remaining in contact with the rest of the peritoneum. The glycosurias were: 0, 1.08, 0, 0, 2.66, 0.57, 0.7, and 0 per cent, with an average of 0.63, which is less than one-third the average of the glycosurias obtained when the painted pancreas remained unisolated from the peritoneum. These figures indicate that the increases

TABLE IV.

Pancreas Isolated; Adrenalin Applied to Pancreas.

Experiment No.	Weight. Sex.	Part and proportion of entire pancreas painted.	Amount of adrenalin (1:1,000 solution).	Blood sugar. Before painting. Before ether.	Blood sugar. Before painting. During ether.	Blood sugar. After painting. Time.	Blood sugar. After painting. Percentage.	Urine. Length of period.	Urine. Amount.	Urine. Sugar.	Albuminuria.	Anesthesia.	Temperature of dog.	Remarks.
	kg.		*cc.*	*per cent*	*per cent*	*hrs. min.*	*per cent*	*hrs. min.*	*cc.*	*per cent*			*°C.*	
											None before.			
13	13.45	One-third (posterior free end).	2.0	0.10	0.11	4	0.12					Ether blown		Free end of pancreas put through
						17	0.13	18	4.4	Tr.	+	through	38.6	a hole in rubber
	Male.	Both sides				→ 4 20	0.11	3 57	75	"	+	cone for 63	39.7	membrane which
		stripped.				22	0.10	17 44	>140	0	+++	min.	38.8	protected the viscera. Vessels leading to tip ligated. Each side painted six times.

													None before.			
14	8.75	One-seventh (posterior free end).	1.5	0.13	0.17	→	10	0.21		11	2.4	Tr.	+	Ether blown through cone for 73 min.	38.9	No blood vessels tied. Pancreas brought outside peritoneal cavity as a loop and other viscera protected from adrenalin flowing on them, by several layers of rubber tissue. Each side painted about seven times.
						2	15	0.12	1	53	6.2	1.08	+		38.2	
	Male.	Both sides stripped.				4	7	0.12	2		15.4	0	+		39.4	
						22	50	0.11	18	50	>100	0	+		39.5	
													None before.		38.4	
15	10.75	One-fifth (posterior free end).	1.5	0.11	0.13	→	10	0.15		11	1.8	0	+	Ether blown through cone for 45 min.	38.6	The procedure same as in No. 14. Each side painted six or seven times.
						2	32	0.11	2	15	44	0	+		38.5	
	Male.	Both sides stripped.				4	18	0.11	1	45	60	0	+		39.2	

TABLE IV—*Continued.*

Experiment No.	Weight. Sex.	Part and proportion of entire pancreas painted.	Amount of adrenalin (1:1,000 solution).	Blood sugar. Before painting. Before ether.	During ether.	After painting. Time.	Percentage.	Urine. Length of period.	Amount.	Sugar.	Albuminuria	Anesthesia.	Temperature of dog.	Remarks.
	kg.		cc.	per cent	per cent	hrs. min.	per cent	hrs. min.	cc.	per cent			°C.	
											None before.			
16	12.0	One-fifth (posterior free end).	1.5	0.12	0.15	→ 11	0.21	13	1.4	0	+	Ether blown through cone for 64 min.	38.7	The procedure same as in No. 14. Each side painted three times.
								1 52	10	Tr.	+		37.9	
	Male.	Both sides stripped.				4 52	0.12	2 36	27	0	+		38.8	
											None before.		39.5	
17	11.5	One-third (posterior free end).	1.5	0.09	0.18	→ 10	0.20	14	1.2	About 1	+	Ether blown through cone for 58 min.	37.1	The procedure same as in No. 14. Each side painted four or five times. Vomited during experiment. Pulmonary edema and pneumonia.
						1 24	0.13	58	9	2.66	+		38.5	
	Male.	Stripped very little.				19 25	0.13	18	>500	0	Tr.		39.0	

											None before.		39.2	
18	9.5	One-half (posterior	1.5	0.12	0 25	→ 11	0.26	16	3	0 0	+	Ether blown	37.4	Procedure similar
		free end and				2 16	0.16	1 55	16 4	0 57	+	through	38.3	to that for No. 14,
	Male.	part of middle).				3 53	0.13	1 35	13	Tr. (?).	+	cone for 66	37.0	except that the
		Stripped well.				21 40	0.10	18	552	0 0	+	min.		duodenum, wrapped in rubber tissue, was also brought outside. Each side painted three times. Dog dying next morning.
											+ before.			
19	12.5	One-third (posterior free end and	2.0	0 12	0 21	→ 14	0.25	33	4	About 0 7	+	Ether blown	37 0	Procedure similar
						3 17	0 12	50	60	Tr.	+	through	38.2	to that for No. 18,
	Male.	part of middle).				5 10	0.12	1 52	55	0 0	Tr.	cone for 73	38.6	except that other
		Stripped very little.				23 30	0.15	18	642	0.0	"	min.	38.9	abdominal viscera were still better protected. Each side painted four times.
											+ before.			
20	11.35	One-half (posterior	2 0	0.13	0 20	→ 10	0-27	21	1.5	0.0	Bloody.	Ether blown	36.1	Procedure similar
		free end and				2 39	0.12	2 20	2.8	0.0	+	through	38.1	to that for No. 19.
	Male.	part of middle).				4 44	0.10	2 5	3.4	0 0	+	cone for 55		Each side painted four times.
		Stripped well.				22 40	0.10	18	>1,000	0.0	+	min.	37.3	

TABLE V.

Pancreas Isolated; Adrenalin Administered Intraperitoneally.

Experiment No.	Weight. Sex.	Amount of adrenalin (1:1,000 solution).	Blood sugar. Before injection. Before ether.	Blood sugar. Before injection. During ether.	Blood sugar. After injection. Time.	Blood sugar. After injection. Percentage.	Urine. Amount.	Urine. Sugar.	Anesthesia.	Remarks.
	kg.	*cc.*	*per cent*	*per cent*	*hrs. min.*	*per cent*	*cc.*	*per cent*		
21	11.5	2.0	0 12	0.26	15	0.32	1.4	About 1	Ether blown through cone for 3 hrs., 42 min.	Pancreas and duodenum lifted out of peritoneal cavity by loops of tape and wrapped in cloths soaked in warm saline solution, adrenalin injected below.
					35	0.29	9.8	5.33		
	Female.				1 3	0.23	8 4	5.33		
					2 4	0.22	3.4	6.00		
					→ 3 4	0.16	4.0	2.85		
					3 39	0.13	4 0	1.48		
22	9.3	2.0	0.12	0.23	15	0.27			Ether blown through cone for 3 hrs.	Procedure similar to that for No. 21. Vomited while under ether.
					46	0.27	0.8	0		
	Female.				→ 1 46	0.28				
					2 46	0.18	1.0	0		
23	10.35	2.0	0.11	0.18	15	0.16			Ether blown through cone for 2 hrs., 7 min.	Procedure similar to that for No. 21.
					45	0.13	5	Tr.		
	Male.			30 min. later 0.15	→ 1 47	0.12	A few drops.	0.5 or less.		
					2 47	0.11	1	0.5 " "		

in glycosuria and hyperglycemia observed after intraperitoneal injections are not of pancreatic origin, the effects of the painting being much smaller when the organ is isolated from the peritoneum. This conclusion becomes even more evident when one examines the results of the few experiments given in Table V, in which adrenalin was injected intraperitoneally while the pancreas was isolated.

The rise in blood sugar in this table is: 0.06, 0.05, and 0.01 per cent, the average being 0.04 per cent. The glycosurias are: 6.0, 0.0, and 0.5 per cent, the average being 2.2 per cent. The number of these experiments is too small to permit of a definite decision, yet the conclusion is in harmony with that drawn from the experiments of Table IV; namely, that the hyperglycemia and glycosuria observed after intraperitoneal injections are not of pancreatic origin.

For the sake of clearness the averages of the amounts of urine and of blood sugar noted in Tables I to V are presented in Table VI.

TABLE VI.

Averages of Glycosuria and Rise of Blood Sugar Due to the Application of Adrenalin.

Averages.	Mode of application.	Glycosuria.	Blood sugar
		per cent	*per cent*
Our 12 experiments (Tables I and II).	Painting unisolated pancreas.	2.23	0.054
Our 8 experiments (Table IV).	Painting isolated pancreas.	0.63	0.035
Our 3 experiments (Table V).	Intraperitoneal injections; pancreas isolated.	2.2	0.04
Vosburgh and Richards' 5 experiments (Table III).	Painting unisolated pancreas.		0.158

DISCUSSION AND CONCLUSIONS.

After Blum's discovery of the production of glycosuria by the subcutaneous injection of adrenal extract, Herter has the merit of having found that injection of adrenalin into the peritoneal cavity also produces glycosuria; this is an undeniable fact. Concerning Herter's claim that intraperitoneal injection gives a higher degree of glycosuria

than subcutaneous or intravenous injection, we offer no comment since we have made no observations on the glycosuric effect of subcutaneous injection of adrenalin, while we have made only three experiments by intraperitoneal injection. The most we can predicate on the basis of the present experiments is that intraperitoneal injection of adrenalin produces a somewhat higher degree of glycosuria than could be anticipated. However, in an earlier study carried out several years ago[6] we arrived at the conception that the more slowly adrenalin was absorbed from the tissues into the circulation, the greater was its glycosuric effect; hence an intramuscular injection, which in its effect is nearly equal to that of an intravenous injection, induced a glycosuria definitely smaller than that set up by a similar dose administered subcutaneously. Unless the absorption from the peritoneal cavity is shown to be different from the absorption from subcutaneous injections, there could be no reason to assume that the glycosuric effect of intraperitoneal injection is much greater than that of subcutaneous injection. We might add that our former experiments do not support Herter's view that subcutaneous injection of adrenalin yields only slight degrees of glycosuria, because it is largely oxidized before entering the circulation. A difference exists in the effects upon blood pressure and upon sugar production, depending upon the mode of administration of adrenalin. With regard to the sugar production, a subcutaneous injection has a definitely greater effect than an intravenous injection; with regard to the blood pressure effect, however, the opposite is true. Herter states that an intraperitoneal injection of adrenalin exerts a smaller effect upon blood pressure than an intravenous injection—a fact which Auer and Meltzer can confirm for the rabbit.[7]

Our experiments lead us to conclusions which do not conform to those of Herter. It will be recalled that Herter and his coworkers state first, that painting the pancreas causes a marked glycosuria and hyperglycemia, and, second, that the glycosuria and hyperglycemia produced by intraperitoneal injections are of pancreatic origin; that is, they are produced by the adrenalin's coming in contact with the pancreas. In our experiments tabulated in Table IV, in which the

[6] Kleiner, I. S., and Meltzer, S. J., *J. Exp. Med.*, 1913, xviii, 190.

[7] Auer, J., and Meltzer, S. J., unpublished observations.

pancreas was isolated from the rest of the peritoneal cavity, the glycosuria was about one-third, and the rise in blood sugar about two-thirds that obtained by painting the unisolated pancreas. Hence two facts may be deduced: first, that the painting of the isolated pancreas produces only mild glycosuria and hyperglycemia, and, second, that the greater production of sugar observed after the painting of the unisolated pancreas cannot be of pancreatic origin. Indeed, our experiments point rather to the conclusion that the larger production of sugar after painting the unisolated pancreas is due to the fact that a large part of the adrenalin escapes to the peritoneum. The last mentioned view is supported by the statement of Herter and Wakeman[8] that "applications to the kidney are apt to yield more sugar than similar application to the liver, intestine, spleen, or brain, but the glycosuria is less marked than after the pancreas has been painted." Emerson and one of us had shown that a dissolved substance painted upon a kidney with an intact membrane is incapable of penetrating the membrane and affecting the kidney, or even incapable of entering the circulation, except when the solution escapes to other parts of the peritoneum.[9] It was this observation which led to the suggestion that the effects observed by Herter of painting the pancreas might have been due to the escape of adrenalin to the celiac ganglion. This point has not been directly tested, but several experiments were performed in which the adrenals were painted with the effect on sugar production apparently as intense as that obtained by painting the unisolated pancreas. However this may be, and whether the production of sugar after painting the unisolated pancreas is due to the escape of adrenalin to some definite organ covered by the peritoneum (celiac ganglion or adrenals) or whether the peritoneum as a whole is responsible for the sugar production, it appears that, when sugar production follows the intraperitoneal injection of adrenalin, it is not of pancreatic origin.

[8] Herter and Wakeman, *Tr. Assn. Am. Phys.*, 1902, xvii, 578, foot-note.

[9] Emerson and Meltzer, S. J., cited in *Tr. Assn. Am. Phys.*, 1902, xvii, 595.

THE SPIROCHETAL FLORA OF THE NORMAL MALE GENITALIA.

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PLATES 30 TO 32.

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An accurate knowledge of the varieties of spirochetal organisms which normally inhabit the smegma or the mucous membranes of the urogenital region has become imperative for the establishment of an etiological relationship between a spirochete and a disease in which the organism may be found in the urine.

The classic work of Inada, Ido, Hoki, and others[1] on the presence of *Leptospira icterohæmorrhagiæ* in the urine of convalescents from infectious jaundice has introduced a new procedure by which the disease may be easily diagnosed, and it is natural that a similar procedure should be followed in the search for an etiological agent in other diseases of infectious origin.

Attention has been directed by Martin,[2] Nankivell and Sundell,[3] and Patterson[4] to the urine in cases of trench fevers. In fact, Nankivell and Sundell early demonstrated minute spirochetes in specimens of urine from soldiers suffering from so called trench fever. Of 26 patients, most of them suffering from a "five-day fever," 99 specimens were examined, with 29 positive findings. Spirochetes were found in 12 out of 15 typical cases, while none of the 8 controls showed a spirochete (Fig. 32). The investigators considered the possibility of contamination of the urine from the smegma or from preputial sources, but it seemed to

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[1] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., Etiology, mode of infection, and specific therapy of Weil's disease (spirochætosis icterohæmorrhagica), *J. Exp. Med.*, 1916, xxiii, 377.

[2] Martin, C. J., quoted by Nankivell and Sundell.[3]

[3] Nankivell, A. T., and Sundell, C. E., On the presence of a spirochæte in the urine in cases of trench fever, *Lancet*, 1917, ii, 672.

[4] Patterson, S. W., Preliminary note on spirochætes occurring in the urine in cases of "P. U. O.," *J. Roy. Army Med. Corps*, 1917, xxix, 503; Spirochætes occurring in the urine in cases of "pyrexia of unknown origin," *Brit. Med. J.*, 1917, ii, 418.

them improbable that the spirochetes came from these sources, since the urine usually contained the minute spirochetes unaccompanied by the coarse *Spironema refringens*, and in case of contamination the presence of the large varieties was to have been expected. Moreover, in some positive instances no spirochetes could be discovered in the smegma. The occurrence of spirochetes in the urine was not constant, that is, not detectable on successive occasions, but was recurrent at irregular intervals. A certain relation seemed to exist between the appearance of spirochetes in the urine and the height of pyrexia, spirocheturia occurring usually 24 hours after the height of fever. In still other cases they appeared on the 14th to the 16th day. They were actively motile, averaged $8.15\,\mu$ in length and $0.3\,\mu$ in width, with an average of five curves; *i.e.*, varying from two and a half curves in $5\,\mu$ to ten curves in $12.5\,\mu$. The spirals varied in depth. The extremities tapered to sharp points with a flagellum at one or both ends. The organism differed from *Treponema pallidum* in its shortness and its fewer spirals.

Patterson, using the Fontana, Wilmaers-Renaux,[5] and India ink methods, examined specimens of urine from various groups of trench affections, 3 cases of trench nephritis, 1 case of pyelonephritis, with abscess of the lungs, 15 cases of relapsing type of pyrexia of unknown origin, 1 case of myalgia following pyrexia of unknown origin, and 5 cases of appendicitis (?) not yet diagnosed, finding spirochetes with the following features: They were about one to one and a half times the diameter of a red blood corpuscle, very thin, with tapering extremities, some having five to eight more or less regular curves, some being straight, some bowed, or lying in a semicircle (Fig. 33). The spirals were not so fine as those of *Treponema pallidum* or so coarse as those of *Spironema recurrentis*. The organisms took Giemsa's, Leishman's, or Romanovsky's stain poorly but were easily demonstrated by the Indian ink method. Patterson depicts the spirochetes found in the abdominal type of cases as rather closely wound, short, thick forms, and those found in the relapsing type as much more tightly coiled. Little attention was given to control cases.

The main objection to the work of the British investigators has been the possibility of an accidental contamination of the urine by unclean surroundings. Many have insisted upon the necessity of collecting specimens by catheterization. The investigation of Stoddard[6] brought out an unsuspected source of spirochetes in the periurethral as well as the intraurethral region of the male genitalia. After examining 50 healthy soldiers and 50 miscellaneous hospital patients without history or symptoms of relapsing fever (trench fever), Stoddard drew the conclusion that (1) spirochetes are not uncommon organisms in the urethra of men without history or symptoms of relapsing fever; (2) many dif-

[5] Wilmaers, L., and Renaux, E., Quarante-sept cas de Spirochétose ictero-hémorragique, *Arch. méd. Belges*, 1917, lxx, 115, 207.

[6] Stoddard, J. L., Occurrence of spirochetes in the urine, *Brit. Mel. J.*, 1917, ii, 416.

ferent varieties are found; (3) some of the varieties seen are morphologically closely similar to pathogenic varieties; (4) the spirochetes occur so definitely within the urethra that they are an obvious source of contamination in uncatheterized specimens of urine; (5) they are a sufficiently dangerous source of error even in catheterized specimens to deserve attention in careful work; and that finally (6) it is possible that a staining reaction or some other morphological character may be discovered to differentiate microscopically the common and harmless from the pathogenic spirochetes. Of 50 hospital cases 56 per cent showed spirochetes, of which 46 per cent were not *Spironema refringens*. Of 50 American soldiers spirochetes in the urethra occurred in 22 per cent, 2 per cent of which showed *Spironema refringens* also. Films from periurethral parts contained more of the coarse *refringens* type.

The spirochetes found by Stoddard measured from 3.75 to 22 μ, more commonly 6.75 to 9 μ, but 11 μ was not uncommon. They were either moderately thick or extremely slender. The ends tapered or were blunt and formed a hook. The spiral length varied from 0.5 to 1 μ. Flattened and longer spirals also occurred, averaging 1.5 to 3.3 μ and as long sometimes as 4 μ. Stoddard states that a type with about eleven curves in 7.5 μ occurs frequently, but I have not been able to verify this finding. In some cases the spirals were exceedingly close and fine and almost impossible to count. They were often irregular, the deep narrow, deep wide, flat narrow, and flat wide types of spirals sometimes occurring simultaneously in the same organism. The middle portion sometimes had looser coils or none at all. In one film many different varieties were often present, including frequently organisms similar to *Leptospira icterohæmorrhagiæ*.[7]

Obviously it is not simple to interpret what one sees in the rich spirochetal material as described by Stoddard, who sees in it many different varieties, including the leptospira type. But, as this article is intended to show, a critical analysis of the spirochetal flora reduces the number of varieties to not more than three, or at most four; namely, *Spironema refringens*, *Treponema calligyrum*, and *Treponema minutum, n. sp.*

Since the time of Schaudinn and Hoffmann a coarse spirochete designated by them *Spirochæta refringens* has been known to inhabit the genital region, but no particular attention has been given to the possibility of the existence of other varieties. It was not until the subject was taken up not only from the morphological but also from the cultural standpoint that some interest came to be attached to these

[7] Noguchi, H., *Spirochæta icterohæmorrhagiæ* in American wild rats and its relation to the Japanese and European strains. First paper, *J. Exp. Med.*, 1917, xxv, 755.

spirochetes. In the present work a strain of *Spironema refringens* was isolated and its morphological and cultural features studied, thus establishing its entity as a species. Later a strain of spirochete (*Treponema calligyrum*) was obtained from a condyloma, which resembled *Treponema pallidum* on the one hand and *Spironema refringens* on the other, being an intermediary organism in its morphological and cultural characteristics. Subsequent observations have led me to regard this particular species as one of the most common varieties that are found in the flora of the smegma or of the urethral region. In fact, *Treponema calligyrum* is more frequently met with than the better known coarse *Spironema refringens*. There is, in addition to these two varieties, another, much smaller spirochete in the genital flora, which will be described in a subsequent paragraph. These three, the minute, medium, and coarse types, constitute the spirochetal flora which at first glance present such a complex aspect.

The smegma and urethral films from six soldiers who were admitted to the Hospital of The Rockefeller Institute for treatment for pneumonia have been examined.[8] The specimens were examined in fresh condition under the dark-field microscope and also as stained preparations. For staining methods Giemsa's stain, Fontana's silver impregnation,[9] and occasionally Benians' Congo red negative im-

[8] These specimens were obtained through the courtesy of Captain Henry T. Chickering.

[9] (*a*) Fix the air-dried film in Solution 1, which consists of

	cc.
Glacial acetic acid	8
Formalin	20
Distilled water	100

for 1 minute and wash well with water. (*b*) Mordant with Solution 2, which consists of

Tannin	5 gm.
Phenol	1 cc.
Water	100 "

for 1 minute over a gentle flame to the point of steaming, then wash thoroughly in water. (*c*) Treat in a 0.25 per cent silver nitrate solution to which one drop of ammonia is added to 40 cc. of the solution. The film turns brown in a few minutes. Wash in water and then (*d*) cover with the mordant and warm it over a flame until it begins to steam. Then wash the film in water and dry.

pression method were employed;[10] also a mordant staining recommended by me for various spirochetes, including *Treponema pallidum*. The method is similar to that advanced by Wilmaers and Renaux, but seems to give a better color value on account of the use of gentian violet instead of fuchsin. The film is fixed in methyl alcohol for 15 minutes, then after being washed in water is covered with a solution of mordant (5 per cent tannin plus 1 per cent phenol) and held over a gentle flame for 1 minute, during which time it begins to steam. It is again washed in running water, covered with a strong aqueous solution of gentian violet to which 1 per cent phenol has been added, and steamed briefly over a flame, then washed well in water, air-dried, and examined. This method gives excellent results also with the *pallidum*. Care must be taken not to make too thick a film.

The number of cases examined was small, but the finding was such that it was sufficient to determine the average flora in male genitalia. The varieties of spirochetes encountered in most of the smegma were the same as those found in one. All contained *Treponema calligyrum* and *Treponema minutum, n. sp.*,[11] and most of them *Spironema refringens*, although the latter was absent in some cases.

No spirochetes were found in the films made from the urethral mucosa by means of a platinum loop. Just where the fault in the technique lay I am unable to explain. The finding was uniformly negative also with the specimens of urine from ten soldiers. With the idea that in nephritis cases there might be more possibility of encountering spirochetes in the urine, ten different specimens from acute as well as chronic cases of nephritis were subjected to a careful examination, but with no positive finding as yet.[12] In Table I

[10] A few drops of a 2 per cent Congo red solution (filtered) are mixed with a drop of the material suspected of containing a spirochete and spread over a clean slide to form a film. The slide after being air-dried is immersed in a jar of absolute alcohol containing 1 per cent hydrochloric acid. In a few minutes the red color of the film turns to a bluish tint. The slide is then removed from the acid alcohol and air-dried.

[11] Noguchi, H., Morphological characteristics and nomenclature of *Leptospira* (*Spirochæta*) *icterohæmorrhagiæ* (Inada and Ido), *J. Exp. Med.*, 1918, xxvii, 575.

[12] The specimens used in these tests were obtained through the courtesy of Dr. W. W. Palmer of the Presbyterian Hospital.

are recorded some of the results obtained in the present study. There are at least three different varieties distinguishable in the photomicrographs or under the dark-field microscope, a minute (*minutum*), a medium (*calligyrum*), and a large (*refringens*) type. Their biometric characteristics, as encountered in twenty-five specimens of each type, are given in Table I.

TABLE I.

Type.	Length.		Thickness.	Spiral amplitude and intervals.	Spiral depth.	No. of spirals or waves.
	Average.	Extremes.				
Minute type.	7–10μ	3–14μ	0 25–0.3μ	0.9–1μ. Fairly regular intervals.	0.2–0 5μ. Some may reach 1μ in penultimate spirals.	7–10 spirals; vary according to length.
Medium type.	9–12μ	4–14μ	0.35–0.4μ	1.75μ. Usually fairly regular; that is, a given amplitude is well maintained in a specimen.	0.5–1μ. Often flattened near the middle in stained specimens. Reaches 1.5μ in some.	5–8, varying according to the spiral amplitude; some only 3.
Large type.	12–16μ	7–22μ	0.7μ	2–3μ. Usually more or less regular.	0.5–1.5μ. Almost constant; in live specimens changing the position of the waves. In stained specimens often irregularly flattened out.	3–5; quite variable; exceptionally 8 in a very long specimen.
T. pallidum.	8–14μ	6–18μ	0 25–0.3μ	1μ	0.8–1μ	8–14; some 16.

Some of the dark-field, as well as the ordinary photomicrographs, representing the minute, the medium, and the large types are shown in Figs. 1 to 14. The minute type is decidedly smaller than *Treponema pallidum* and has a larger number of shallower spirals in proportion to its length (Figs. 1 and 5). There are also many short specimens such as are never found among the *pallida.* The medium type has an aspect like that of atypical specimens of the *pallidum.* The spirals are fairly deep but not so deep as those of a typical *pallidum,* while the intervals between them are wider (Figs. 2, 5, 6, 9, and 10). All appear somewhat thicker than the *pallidum* (Fig. 21) when seen under the dark-field microscope. This does not apply to the specimens stained by mordanting techniques (Fontana's and the writer's), in which there occurs often an uncontrollable uneven heavy deposit of the dyes, due to various external factors (Figs. 5 to 14, 17, 20, and 22). Among organisms of the medium type are noticed two forms, one with more closely set spirals and the other with wider ones, but this is due to certain temporary conditions and may be made to disappear or reappear by regulation of cultural conditions. For example, there will be more of the wide, flat spiral forms when the medium is more fluid. The large type is much heavier, comparatively short, with few spirals, and constantly changes its curves (Figs. 4 and 8). The spirals of the minute type become readily obliterated after the death of the organism (upper organism in Fig. 8).

When fresh they all exhibit moderately active movements, rotary, lashing, and forward and backward locomotion. The large type is the most energetic and the minute variety the least so. In many of the large type there is a distinct double contour effect upon examination under the dark-field microscope. All are provided with a terminal filament or flagellum at one or both ends.

As has been noted before, not all smegmata contain a spirochete, and the varieties present may all belong to one or two of the three groups. As a rule, however, all three types are present, the medium type usually predominating.

Cultural Characteristics.

By selecting the smegma specimens which were rich in the type desired, a culture of each of the three types described was obtained. The technique employed was similar to that previously used for the cultivation of *Spironema refringens*[13] and *Treponema calligyrum*.[14] All require strict anaerobiosis (addition of fresh tissue to the media), the presence of suitable body fluid (ascitic fluid), and an optimal temperature (37°C.). The growth in the fluid medium, consisting of ascitic fluid and a piece of fresh rabbit kidney and a layer of paraffin oil, is invisible, while in a solid medium, consisting of 2 parts of the neutral agar and 1 part of ascitic fluid with a piece of the fresh rabbit kidney at the bottom, a faint haze appears to develop near the tissue, gradually extending upward within a fortnight. No discrete, circumscribed, sharp colonies have so far been observed. In this respect all the strains obtained are analogous to the cultures of various anaerobic treponemata and spironemata.[15] None produced a putrefactive or offensive odor, the absence of odor from the culture of the minute type serving to distinguish it from either *Treponema microdentium*[16] or *Treponema mucosum*.[17] Carbohydrates added to the culture media exert neither a favorable nor a retarding influence upon growth, and no visible alterations of the media result from their presence.

In young fluid cultures, whether of the minute, medium, or large type, the organisms are short and active, but as they grow older (2 weeks) the longer forms, some in chains, and some in tangled masses, predominate, their motility meanwhile being considerably reduced. The spirals are quite regular (Figs. 23 to 31). Very short forms do

[13] Noguchi, Pure cultivation of *Spirochæta refringens*, *J. Exp. Med.*, 1912, xv, 466.

[14] Noguchi, Cultivation of *Treponema calligyrum* (new species) from condylomata of man, *J. Exp. Med.*, 1913, xvii, 89.

[15] Noguchi, Experimental research in syphilis with especial reference to *Spirochæta pallida* (*Treponema pallidum*), *J. Am. Med. Assn.*, 1912, lviii, 1163.

[16] Noguchi, Cultural studies on mouth spirochætæ, *Treponema microdentium* and *macrodentium*, *J. Exp. Med.*, 1912, xv, 81.

[17] Noguchi, *Treponema mucosum* (new species), a mucin-producing spirochæta from pyorrhea alveolaris, grown in pure culture, *J. Exp. Med.*, 1912, xvi, 194.

not appear in the solid media, the organisms appearing to attain average length within a short time. The spirals are remarkably regular in solid media and so deep, in the case of the medium type, as to simulate a *pallidum* (Fig. 29). In older cultures two, three, and four individuals in chains have occasionally been encountered (Fig. 27). Division in all three types is brought about by transverse and perhaps also by longitudinal fission.

Identification.

The morphological and cultural characteristics of the large type show it clearly to be a *Spironema refringens*, those of the medium type identify it with *Treponema calligyrum*. The latter type may be the same organism as that described by Levaditi and Stanesco[18] in 1909 as *Spirochæta gracilis*, found in a case of balanitis, but, as pointed out previously, these authors used a name already designating another spironema from an ulcerating jaw, which is very different from the present medium type. The name *Treponema calligyrum* was given to a non-pathogenic spirochete cultivated from the surface of a condyloma, but subsequent studies on the spirochetal flora of the genitalia have convinced me that this type is one of the most commonly met inhabitants of the genital region.

The minute type is not unlike the minute spirochete of the mouth, *Treponema microdentium*, but its cultural characteristics differentiate it from the latter. *Treponema microdentium* produces a peculiar odor, especially when freshly isolated, and in a fluid medium the color of the fresh tissue is made grayish within about 10 days and the fluid somewhat faintly opalescent. The *minutum* produces no odor and remains without any perceptible action upon the culture medium, though in dimension there is a general resemblance.

In order to determine whether these two closely similar organisms are immunologically related to each other, agglutination tests were undertaken in which the action of a *microdentium* antiserum (rabbit) was tested on both types. It was found that the serum caused a marked agglutination of *Treponema microdentium* in 1:500 dilution but only a slight one with two different strains of the *minutum*, even

[18] Levaditi, C., and Stanesco, V., Culture de deux spirochètes de l'homme (*Sp. gracilis* et *Sp. balanitidis*), *Compt. rend. Soc. biol.*, 1909, lxvii, 188.

in a dilution of 1:20. In this connection it may be mentioned that a *calligyrum* serum (rabbit) gave a copious agglutination with the cultures of the medium type in a 1:200 dilution, but only a slight one with those of the minute type in 1:20. There was a partial reaction, but not marked enough to render the differentiation of the two types difficult.

In all probability the minute smegma spirochete has been repeatedly observed by investigators, but no special attention seems to have been given to its identity. I have been accustomed to pass it over as probably identical with *Treponema microdentium*. Now that this type has been found to constitute an independent group, differentiated by several well defined features, it may well be known under a separate name, *Treponema minutum*.

In the spirochetal flora of male smegma, therefore, only the three forms, *Spironema refringens*, *Treponema calligyrum*, and *Treponema minutum*, were recognized.

DISCUSSION AND SUMMARY.

The varieties of spirochetes enumerated and photomicrographed from the male smegma flora represent practically every form hitherto described by Nankivell and Sundell and by Patterson in the specimens of urine from trench fever cases (Figs. 32 and 33). The urethral flora, as studied by Stoddard, seem to contain more varieties, but, except those of his more detailed morphological descriptions, every form observed by him is among those found in the smegma. Stoddard saw certain forms with hooked ends suggestive of the *Leptospira icterohæmorrhagiæ* of infectious jaundice, but the resemblance ends with this one feature, and differentiation should always be possible under the dark-field microscope, by means of which the leptospira reveals its highly characteristic minute elementary spirals, presenting the appearance of a chain of dots (Fig. 18). Fig. 19 shows that a very favorable fixation with the osmic acid vapor followed by Giemsa's staining may also bring out the elementary spirals. Of all the spirochetes, none has so closely set spirals as the jaundice leptospira, the distance between two spirals being only 0.5 μ. Various methods, including Fontana's, Benians', the mordant gentian violet stain, or Burri's India ink method, are inadequate

to differentiate the leptospira from other spirochetes (Figs. 12, 14, 15, 16, 17).

Why a positive spirochete finding with the films from the urethra and in the specimens of urine was not obtained, is difficult to explain, except on the grounds of the paucity of specimens examined. At all events, the recent negative results reported by Fiessinger[19] with French soldiers and invalids after cleansing of the urethra and glans seem to be in harmony with my results.

In conclusion it may be stated that *Spironema refringens*, *Treponema calligyrum*, and *Treponema minutum* represent practically all the spirochetal forms observed in the male smegma flora. A leptospira has never been conclusively shown to be present in the specimens of normal urine or smegma. For the satisfactory microscopic demonstration of a leptospira a dark-field illuminator is indispensable.

EXPLANATION OF PLATES.

Plate 30.

Magnification, × 1,000.

Figs. 1 to 4. Dark-field views of the spirochetes in a male smegma. Fig. 1 represents *Treponema minutum*, Fig. 2 *Treponema calligyrum*, Fig. 3 *Spironema refringens*, and Fig. 4 a *Spironema refringens* (below) and a *Treponema minutum*.

Figs. 5 to 11. Various types of spirochetes in smegma, stained by Fontana's method.

Fig. 5. Two specimens of *Treponema minutum*.

Fig. 6. A specimen of *Treponema minutum* and two of *Treponema calligyrum*, of varying lengths.

Fig. 7. A group of *Treponema calligyrum*, with two specimens of *Treponema minutum*.

Fig. 8. A group of *Spironema refringens* from a sample of male smegma.

Figs. 9 to 11. *Treponema calligyrum* from two different specimens of male smegma.

Figs. 12 and 13. *Treponema calligyrum* in preparation stained by the mordant gentian violet method. In Fig. 12 there are two specimens without distinct spirals which closely resemble *Leptospira icterohæmorrhagiæ* in similar stained preparations. Further study by means of a dark-field microscope is necessary to determine whether they are leptospira or *calligyrum*.

Fig. 14. A group of *Treponema calligyrum* type from a specimen of male smegma, stained by Giemsa's method. The organisms appear much thinner here than in specimens stained by other methods. A hooked spirochete resembling strongly the leptospira is seen near the left upper corner.

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[19] Fiessinger, N., À propos des Spirochètes du méat et de l'urine de l'homme normal, *Compt. rend. Soc. biol.*, 1918, lxxxi, 38.

FIGS. 15 to 19. *Leptospira icterohæmorrhagiæ* under various conditions (for comparison).

FIG. 15. Four specimens of *Leptospira icterohæmorrhagiæ* stained by the mordant gentian violet method. They appear blunt and curved and without any indication of the minute elementary spirals which are the characteristic feature of this genus. As they appear here they are indistinguishable from the stretched forms of the *calligyrum* type.

FIG. 16. A few leptospiræ as demonstrated by Benians' Congo red method. Here, too, they do not show their elementary spirals.

FIG. 17. A group of *Leptospira icterohæmorrhagiæ* from a culture, stained by Fontana's method. They fail to show their elementary spirals by this staining.

FIG. 18. A leptospira viewed under the dark-field microscope, showing its minute elementary spirals.

FIG. 19. A number of *Leptospira icterohæmorrhagiæ*, fixed with osmic acid vapor and stained by Giemsa's stain, showing the elementary spirals.

FIGS. 20 to 22. *Treponema pallidum* under different conditions (for comparison).

FIG. 20. *Treponema pallidum* when stained by the mordant gentian violet method.

FIG. 21. *Treponema pallidum* under the dark-field microscope.

FIG. 22. *Treponema pallidum* as stained by Fontana's silver impregnation method.

PLATE 31.

Magnification, × 1,000.

FIGS. 23 and 24. Dark-field view of a culture of *Treponema minutum* from a male smegma.

FIG. 25. *Treponema minutum* from a culture. Stained by the mordant gentian violet method.

FIG. 26. Similar specimens stained by Fontana's method.

FIG. 27. Dark-field view of a culture of *Treponema calligyrum* from a male smegma.

FIG. 28. A culture of *Treponema calligyrum* stained by the mordant gentian violet method.

FIG. 29. Similar specimens stained by Fontana's method.

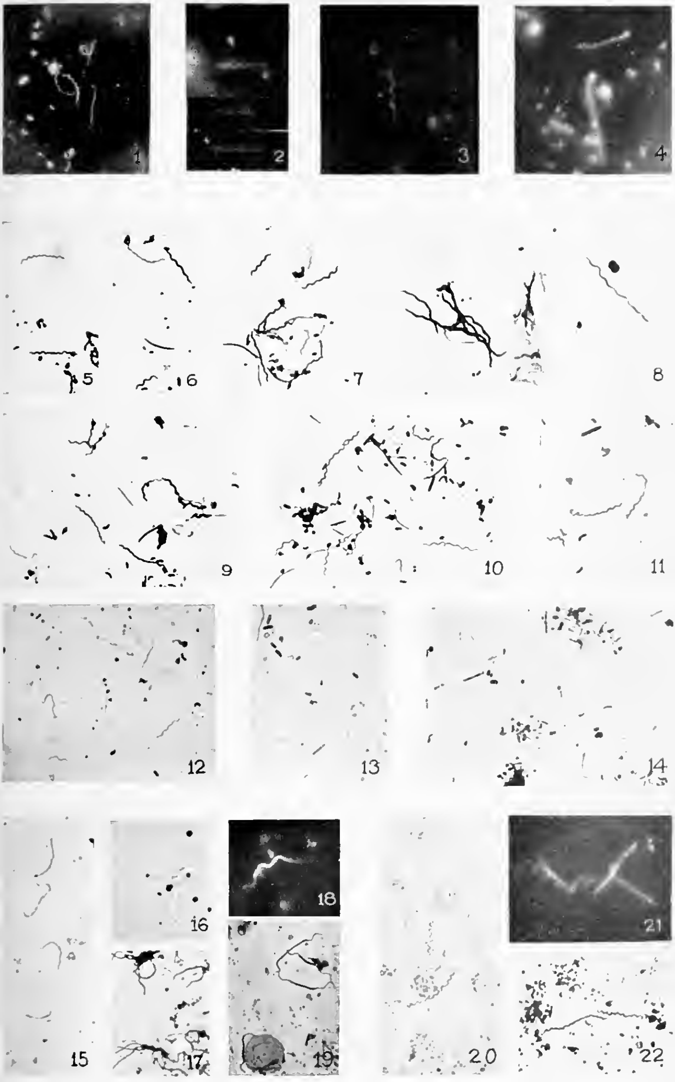
FIG. 30. Dark-field view of a culture of *Spironema refringens* from a male smegma.

FIG. 31. Similar specimens stained by Fontana's method.

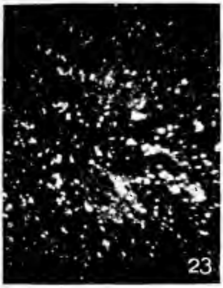
PLATE 32.

FIG. 32. Photographic reproduction of the photomicrographs of spirochetes in Nankivell and Sundell's article on the spirochetes in the urine in trench fever cases.[3]

FIG. 33. Photographic reproduction of the schematic drawings by Patterson in his article.[4]



(Noguchi: Spirochetal flora of normal male genitalia.)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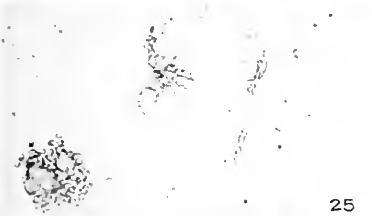
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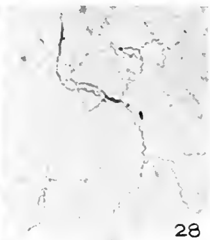
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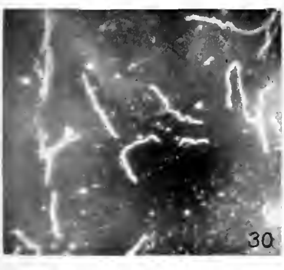
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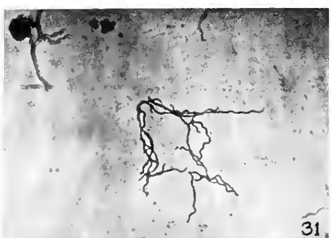
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(Noguchi: Spirochetal flora of normal male genitalia.)

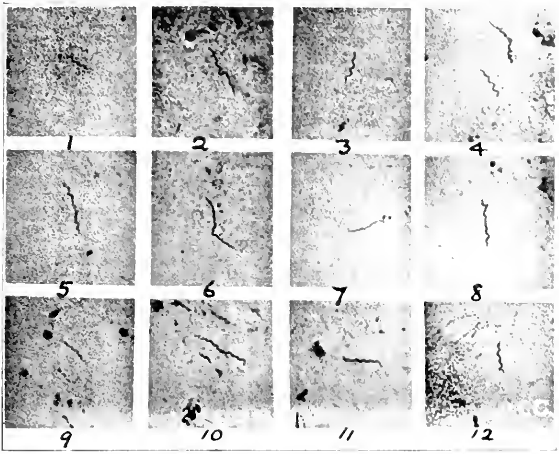
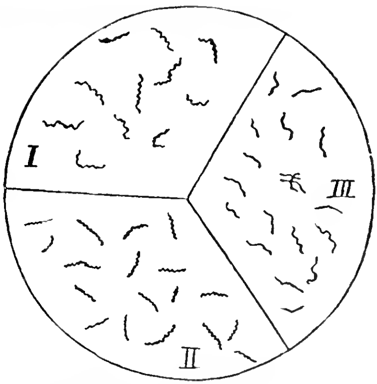


FIG. 32.



I. Spirochetes of Type 1, abdominal P. U. O.
II. Type 2, relapsing P. U. O.
III. Spirillar form from urethra.

FIG. 33.

(Noguchi: Spirochetal flora of normal male genitalia.)

PHYSIOLOGICAL STIMULATION OF THE CHOROID PLEXUS AND EXPERIMENTAL POLIOMYELITIS.

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When the active filterable virus of poliomyelitis is injected into the blood of monkeys, infection and paralysis almost never follow even when the quantity of virus introduced is very large. The reason assigned for the non-infectiousness of the virus under these conditions, compared with the remarkable activity displayed by it when brought into immediate proximity with the central or even the peripheral nervous organs, is the inability of the virus to pass the barrier of the choroid plexus and the blood vessels of the central nervous system. Flexner and Amoss[1] have shown in several series of experiments that when sterile irritating chemical substances are introduced from without and by lumbar puncture into the subarachnoid space, the injury inflicted upon the choroid plexus and blood vessels of the meninges and possibly those of the central nervous organs also, facilitates the passage of the virus from the blood into the nervous tissues under conditions leading to infection, paralysis, and death from poliomyelitic disease. Their experiments have led them to view the meningeal-choroidal complex as constituting in man a defensive mechanism against infection with the virus of poliomyelitis.

According to this view, disturbance of the integrity of the defensive complex arising from any cause would predispose to infection with the virus, provided the disturbance synchronized with the wide distribution of the virus, such as is believed to be the case during epidemics of poliomyelitis. The experiments of Flexner and Amoss have indicated that the qualitative changes in the meningeal-choroidal com-

[1] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249; 1917, xxv, 525. Amoss, H. L., and Eberson, F., *ibid.*, 1918, xxvii, 309.

plex, permitting the escape of the virus from the blood into the nervous tissues, may be almost infinitesimally small. Thus the mere substitution of the cerebrospinal fluid of one monkey for that of another sometimes suffices to open this way. The structural alterations induced by this procedure must be so slight as to be regarded merely as molecular; and yet they have proved adequate to overcome the defensive mechanism.

All the means employed up to the present to disturb the mechanism may be regarded as organic in their effect. Moreover, they have always acted from without, in the sense that they have been brought into relation with the meningeal-choroidal complex through the medium of the cerebrospinal fluid already present in the subarachnoid space. The question presented itself whether a functional effect merely and acting, as it were, from within, might likewise open the way for the passage of the virus from the blood into the nervous tissues. A method was a hand to test experimentally this possibility.

The cerebrospinal fluid is a secretion derived from the blood through the mediation of the choroid plexus. The secreting cells of the plexus exercise a highly precise discrimination in respect to the quality and quantity of the constituents taken from the blood and passed on to the subarachnoid space. The composition of the cerebrospinal fluid not only differs markedly from that of the blood, but dissolved drugs and bacteria and their toxic and other metabolic products present in the blood are capable of being excluded perfectly from the cerebrospinal fluid by the choroidal mechanism.

The stimulus on which the secretory activity of the choroid plexus depends has been shown by Dixon and Halliburton[2] to be a hormone contained within the choroid plexus and to a less extent in the brain substance. The liberation of this hormone into the blood is the precursor to and regulating medium of the choroidal secretory activity, through which the cerebrospinal fluid is elaborated. By increasing experimentally the quantity of the hormone within the blood the amount of cerebrospinal fluid secreted within a unit of time may be increased.

[2] Dixon, W. E., and Halliburton, W. D., *J. Physiol.*, 1910, xl, p. xxx; 1913–14, xlvii, 215; 1914, xlviii, 128.

This phenomenon provides, therefore, a means by which the choroid plexus may be stimulated from within and made to perform its secretory function in an intensified manner. It furnishes a simple method for determining whether merely increased functional activity, independent of structural or organic alterations, suffices to open the way for the passage of the poliomyelitic virus from the blood into the central nervous organs under circumstances leading to infection.

The experiments to be described were carried out to determine this point. The procedure followed for preparing and injecting the extract of the choroid plexus was closely modelled on that of Dixon and Halliburton. These investigators found that an intravenous injection of a saline extract of the choroid plexus, after a delay of a few seconds, causes the cerebrospinal fluid to flow actively for a variable time, after which the flow ceases gradually. The second injection produces little or no effect unless the intervening interval of time is about 10 or 15 minutes.

EXPERIMENTAL.

The starting-point of our experiments was a repetition of the decisive experiments of Dixon and Halliburton. The first step was the preparation of an extract of the choroid plexus. 1 gm. of the dried plexus was ground up with clean sand in a mortar in 100 cc. of isotonic saline solution. The suspension was filtered and the filtrate employed for injection. 5 cc. of the filtrate caused a marked increase in the flow of cerebrospinal fluid in a dog weighing 12 kilos. Extracts of the fresh plexus give an equivalent result, and boiling does not destroy the activity. The active substance is, moreover, soluble in dilute and absolute alcohol. An extract of the brain is less active than one of the plexus. Material from the dog, sheep, or ox may be employed. Dixon and Halliburton, who established these points, express the view that some product of the brain's metabolism passes to the choroid plexus and this hormone stimulates to activity the secreting epithelium covering the plexus. They also discuss the possibility of the hormone's originating in the choroidal epithelium and passing secondarily to the brain tissue. They incline to the first alternative.

Preparation of the Choroid Extract. Isotonic.—Plexuses removed from the fresh brains of sheep under sterile conditions are washed free from blood in sterile saline solution, dried between filter paper, weighed, made up to 1 per cent suspension in isotonic saline solution, ground with sand, and filtered.

Hypertonic.—The same steps are followed except that 10 per cent of the dried plexuses are suspended in 8.5 per cent saline solution. The stock solution is then diluted 1 part to 9 of sterile distilled water before injecting. The filtering of the viscous mixture, which is a slow process, may be substituted by rapid centrifugalization. The clear supernatant fluid is removed and diluted as indicated.

Preliminary Tests.

The technique of the experiments was perfected on dogs in accordance with the method devised by Dixon and Halliburton. A single protocol is appended to illustrate a successful experiment.

Oct. 22, 1917. Dog; weight 12.5 kilos. Anesthesia: chloroform, morphine, and urethane (subcutaneous). Subcerebellar cistern punctured. After the first rapid rush of cerebrospinal fluid was over, the flow was measured in drops per minute and total volume for 10 minutes. In this animal the first rapid escape was 8.2 cc. The slower flow is divided into three 10 minute periods: (*a*) before injecting extract and (*b*) (*c*) after injecting two separate quantities of the extract into the left femoral vein.

1st Period. Before Injection of Extract.

Drops per min.	Total in 10 min. *cc.*
3, 0, 0, 0, 1, 0, 3, 1, 1, 2	0.7
0, 1, 0, 0, 1, 0, 0, 0, 0, 1	0.2

2nd Period. 5 Cc. of Extract Injected into Left Femoral Vein.

Drops per min.	Total in 10 min. *cc.*
0, 5, 3, 2, 1, 1, 1, 1, 1, 1	1.2
1, 0, 0, 3, 3, 0, 0, 0, 1, 1	0.7
1, 0, 0, 1, 0 1, 2, 1, 1, 1	0.5

3rd Period. 3.8 Cc. of Extract Injected into Left Femoral Vein.

Drops per min.	Total in 10 min. *cc.*
2, 1, 0, 1, 1, 0, 1, 1, 1, 1	0.4
1, 1, 0, 0, 1, 0, 0, 0, 1, 0	0.2
0, 0, 0, 0, 0, 0, 0, 0, 0, 1	0.05

The effect of the choroidal extract is observed after each injection, but not so markedly after the second injection.

The preliminary test on monkeys was even more satisfactory. The extract was injected into the basilic vein of a *Macacus rhesus* in an amount of 10 cc. in an animal weighing 3.5 kilos, without producing an observed ill effect. Two protocols of preliminary experiments on monkeys are given.

Oct. 22, 1917. Monkey A, *Macacus rhesus*; weight 5 kilos. Anesthesia: ether, morphine, and urethane. Puncture of subcerebellar cistern. The periods and readings are the same as in the previous protocol. The first flow of fluid following the puncture was 4.4 cc.

1st Period. Before Injection of Extract.

Drops per min.	Total in 10 min. *cc.*
2, 1, 1, 0, 1, 1, 1, 2, 5, 0	0.8
3, 2, 1, 1, 1, 1, 1, 0, 0, 0	0.5

2nd Period. 3.5 Cc. of Extract Injected into Basilic Vein.

Drops per min.	Total in 10 min. *cc.*
0, 0, 2, 2, 1, 1, 1, 1, 2, 2	0.9

Oct. 25, 1917. Monkey B, *Macacus rhesus*; weight 3.5 kilos. Anesthesia: ether, morphine, and urethane. The first flow of fluid following the puncture was 4.1 cc.

1st Period. Before Injection of Extract.

Drops per min.	Total in 10 min. *cc.*
1, 4,* 2, 1, 1, 2, 4,* 3, 1, 1	1.6
2, 0, 0, 0, 0, 0, 0, 0, 0, 0	0.1

2nd Period. 6 Cc. of Extract Injected into Basilic Vein.

Drops per min.	Total in 10 min. *cc.*
1, 1, 1, 1, 1, 1, 1, 1, 1, 1	0.7
1, 0, 0, 1, 1, 0, 1, 1, 1, 1	0.3

* In the interval represented by the two asterisks ether was administered.

The deduction from the three preliminary experiments is to the effect that the choroidal extract which we prepared and employed was an active one. There is another point which may be mentioned here. In order to obtain the stimulating effect of the extract, it

would appear that the animals need to be in good condition. Two monkeys in an advanced stage of tuberculosis showed no effect from the injections.

Experiments with Choroid Extract.

In carrying out experiments with the extract, the quality of the virus is of prime importance. It must be of such a degree of activity that it will not itself induce infection by simple intravenous injection, and yet it must be active enough to cause infection under conditions in which it is enabled to pass the choroidal-meningeal barrier. The importance of this consideration is illustrated by the first protocols.

Experiment 1.—Control A, *Macacus rhesus.* Dec. 5, 1917. Intravenous injection of 40 cc. of centrifugate of 5 per cent emulsion of fresh mixed virus. Dec. 11. Legs weak or paralyzed; right deltoid weak. Dec. 13. Both legs paralyzed; tremor of head. Dec. 14. Prostrate. Dec. 19. Died.

Autopsy.—Lesions of poliomyelitis.

Monkey C, *Macacus rhesus.* Dec. 5, 1917, 12.50 p.m. Intravenous injection of 40 cc. of centrifugate as in control. 12.55, 1.25, and 4.55 p.m. Intravenous injection of choroid extract. Dec. 6, 11 a.m. and 5 p.m. Injection of choroid extract. Dec. 12. Left facial paralysis and slight ataxia. Dec. 14. Legs paralyzed; deltoids weak. Dec. 17. Arms and back weak; lies down. Dec. 19. Improving. Jan. 2, 1918. Recovering use of limbs. Jan. 8. Recovered except for residual paralysis of legs.

Monkey D, *Macacus rhesus.* Dec. 5, 1917, 1.05 p.m. Intravenous injection of 40 cc. of centrifugate as in control. 1.10, 1.40, and 5.10 p.m. Injection of choroid extract. Dec. 6, 11 a.m. and 5.10 p.m. Injection of choroid extract. Dec. 7 and 8. Injection of choroid extract. Dec. 10. Right facial and double deltoid paralysis. Dec. 11. Died.

Autopsy.—Lesions of poliomyelitis.

This experiment is wholly inconclusive as to any promoting effects of the choroidal extract after an intravenous inoculation of the virus. Since the virus was of so high a degree of activity as to induce a fatal infection in the control monkey, the occurrence of paralysis in the other two animals was to be expected. Moreover, a comparison of Monkeys C and D suffices to dissipate any notion that the choroidal extract might have the effect of minimizing the action of the virus since Monkey C partially recovered from the paralysis. Indeed, this experiment is a pertinent illustration of the factor of individuality

in affecting the outcome of an attack of poliomyelitis in the monkey, as well as in man. This factor of individuality appears even more emphatically in the next experiment.

Experiment 2.—Control B, *Macacus rhesus.* Oct. 29, 1917. Intracerebral inoculation of 1 cc. of 5 per cent emulsion of fresh spinal cord and medulla from paralyzed monkey. Nov. 2. Excited; tremor of head. Nov. 3. Tremor increased; ataxic. Nov. 6. Right facial paralysis; left arm weak. Nov. 8. All limbs paralyzed; moribund; etherized.

Autopsy.—Lesions of poliomyelitis.

Control C, *Macacus rhesus.* Oct. 29, 1917. 50 cc. of centrifugate of fresh emulsion of brain and cord, same as Control B, injected intravenously. Nov. 5. Double facial paralysis; ataxia; paralysis of right deltoid. The paralysis extended rapidly so that by evening the animal was prostrate and death occurred during the night.

Autopsy.—Lesions of poliomyelitis.

Monkey E, *Macacus rhesus.* Oct. 29, 1917, 12.50 p.m. Intravenous injection of 50 cc. of centrifugate, same as Control C. 1, 1.30, and 5 p.m. 5 cc. of choroidal extract injected. Oct. 30, 11 a.m. and 5 p.m. 5 cc. of choroidal extract injected. Oct. 31 and Nov. 1. 5 cc. of choroidal extract injected. Nov. 5 and 6. No extract injected. Nov. 7. Ataxia; limbs weak. Nov. 8. Right facial paralysis; deltoids paralyzed; legs weak. Nov. 10. Prostrate. Nov. 12. Moribund; etherized.

Autopsy.—Lesions of poliomyelitis.

Monkey F, *Macacus rhesus.* Treatment identical with that of Monkey E, except that no intravenous injections of choroidal extract were given after Oct. 30. This animal never showed any symptoms and was dismissed from observation on Nov. 19, at which time it was perfectly well.

The only deduction from this experiment is to the effect that the virus was sufficiently active to cause infection and paralysis in two of three monkeys into which it was injected intravenously while a third monkey was sufficiently insusceptible to resist its power of inducing infection. The choroidal extract probably played no essential part in the results.

In order to determine directly whether the choroidal extract exercised a restraining influence on the development of the infection the next experiment was performed.

Experiment 3.—Control D, *Macacus rhesus.* Dec. 4, 1917. Intraspinal injection of 2 cc. of sterile isotonic saline solution. Dec. 5. Intravenous injection of 40 cc. of centrifugate of fresh mixed virus. Dec. 10. Tremor; ptosis. Dec.

11. Ataxia; left deltoid and right leg weak. Dec. 12. All extremities paralyzed; etherized.

Autopsy.—Lesions of poliomyelitis.

Test: Monkey G, *Macacus rhesus*. Dec. 4, 1917, intraspinal injection of saline solution and Dec. 5, 12.05 p.m., intravenous injection of virus as in the control. 12.10, 12.40, and 4.10 p.m. 5 cc. of choroidal extract injected. Dec. 6. Repeated injection of extract at 10.10 a.m. and 4.10 p.m. Dec. 11. Left facial and right leg paralysis. Dec. 12. Both legs paralyzed; arms weak. Dec. 14. All extremities paralyzed. Progressive recovery followed. Animal regained use of arms. Jan. 18, 1918. Died of intercurrent infection.

Autopsy.—Healed lesions of poliomyelitis.

It is obvious that the course of the infection was practically identical in these two animals, and no inhibitory effect of the choroidal extract can be discerned.

Passing now to a virus which is incapable in the quantity employed of inciting infection from simple intravenous injection, we find that the injection of the choroidal extract does not change the results.

Experiment 4.—Control E, *Macacus rhesus*. Nov. 13, 1917. Intravenous injection of 25 cc. of centrifugate of active fresh virus (emulsion of medulla and spinal cord of paralyzed monkey). No symptoms developed and the animal was dismissed from observation on Dec. 17, at which time it was perfectly well.

Monkey H, *Macacus rhesus*. Nov. 13, 1917, 12.55 p.m. Intravenous injection of 25 cc. of virus as in the control. 1, 1.30, and 5 p.m. Injection of 5 cc. of choroidal extract. Nov. 14, 11 a.m. and 5 p.m. Injection of 5 cc. of choroidal extract. Nov. 15 and 16. Injection of 5 cc. of choroidal extract. No symptoms developed and the animal, in perfect health, was dismissed from attention on Nov. 30.

Experiment 5.—Control F, *Macacus rhesus*. Dec. 18, 1917. Intravenous injection of 32.5 cc. of centrifugate of active fresh virus (emulsion of medulla and spinal cord of paralyzed monkey). No symptoms developed.

Monkey I, *Macacus rhesus*. 12 m. Intravenous injection of 32.5 cc. of centrifugate as in the control. 12.05, 12.35, and 4.05 p.m. 5 cc. of choroidal extract injected. Dec. 19, 10.15 a.m. and 4.05 p.m. 5 cc. of choroidal extract injected. Dec. 20 and 21. 5 cc. of choroidal extract injected. No symptoms developed. The animal was dismissed from observation on Jan. 8, 1918, at which time it appeared perfectly well.

DISCUSSION AND SUMMARY.

The experiments recorded in this paper serve, in the first place, to confirm the experiments of Dixon and Halliburton on the stimulating effect of intravenous injections of extracts of choroid plexus in

the secretion of the cerebrospinal fluid, and extend their observations to monkeys.

They bring out also the variable effects of the virus of poliomyelitis, variations affected by the quality of the virus and also by the individual powers of resistance to infection possessed by individual monkeys. These factors of variation must be taken into account in performing and interpreting experiments on infection and particularly those on immunity and specific therapy in relation to poliomyelitis.

In general it may be said that experimental infection by way of the blood is not easy to produce in monkeys unless some contributing factor, such as the existence of a coincident aseptic meningitis, operates at the same time. And yet Experiments 1 and 2 show that when the strength of the virus is great the injection of relatively considerable quantities suffices to induce infection and paralysis, but not in all instances.

The chief outcome of the experiments has been to determine the fact that when the intravenous inoculation of the virus does not in itself suffice to induce infection and paralysis, the intravenous injection of extracts of the choroid plexus, which in themselves excite the secretory functions which preside over the formation of the cerebrospinal fluid, is powerless to modify this result. This fact would seem to be of interest and importance, since it has already been shown that very slight structural changes in the meningeal-choroidal complex suffice to make possible or certain infection under these circumstances. Apparently mere augmentation, from time to time, of the secretory functions of the choroid plexus, through intravenous injection of an extract of the choroid plexus and while the virus is still circulating, is insufficient to insure passage of the virus from the blood into the nervous tissues, upon which infection depends. Neither does the augmentation exercise a restraining influence on the development of infection otherwise capable of taking place.

THE AUTODIGESTION OF NORMAL SERUM THROUGH THE ACTION OF CERTAIN CHEMICAL AGENTS. I.

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INTRODUCTION.

In contrast with the so called defensive ferment, or "*Abwehrferment*" of Abderhalden, which has been recently much studied and discussed, comparatively little attention has been paid to the proteolytic ferment in normal serum, to which only occasional brief references can be found.

Abderhalden[1] has stated that he sometimes found a proteolytic ferment in the sera of guinea pigs and rabbits, which he held to have arisen through the introduction of foreign proteins, such as those due to the ingestion of plants, or to infectious diseases, especially coccidiosis. Stephan[2] reported that guinea pig serum shows an apparently polyvalent proteolytic power. Fuchs[3] found that rabbits inoculated with serum gave a positive ninhydrin reaction with other kinds of substrates, and he explained this result by assuming that the sera of herbivorous animals contain a comparatively large amount of dialyzable substance. Michaelis and von Lagermarck[4] obtained a positive Abderhalden reaction not only with pregnant serum but also with non-pregnant and even male serum, and they came to the conclusion that they could not confirm the existence of the specific ferment in Abderhalden's sense. Van Slyke, Vinograd-Villchur, and Losee[5] also

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[1] Abderhalden, E., Abwehrferment. Das Auftreten blutfremder Substrate und Fermente im tierischen Organismus unter experimentellen, physiologischen und pathologischen Bedingungen, Berlin, 4th edition, 1914, 53–54.

[2] Stephan, R., Die Natur der sogenannten Abwehrfermente, *Münch. med. Woch.*, 1914, lxi, 801.

[3] Fuchs, A., Tierexperimentelle Untersuchungen über die Organspezifität der proteolytischen Abwehrfermente (Abderhalden), *Münch. med. Woch.*, 1913, lx, 2230.

[4] Michaelis, L., and von Lagermarck, L., Die Abderhaldensche Schwangerschaftsdiagnose, *Deutsch. med. Woch.*, 1914, xl, 316.

[5] Van Slyke, D. D., Vinograd-Villchur, M., and Losee, J. R., The Abderhalden reaction, *J. Biol. Chem.*, 1915, xxiii, 377.

found proteolytic ferment in non-pregnant human serum by means of Van Slyke's method of amino nitrogen determination.

The existence, then, in normal human and animal serum, of a non-specific proteolytic ferment which digests certain proteins other than the serum has often been proved, but little investigation into the nature of this ferment has hitherto been made. The question of autodigestion of normal serum has received some attention from a few investigators, Delezenne and Pozerski[6] having observed the autolysis of the serum under the influence of chloroform.

The present paper deals with a phenomenon of the autodigestion of normal serum brought about with certain chemical agents under various conditions.

Materials and Methods of Study.

Guinea pig serum was used chiefly in the present investigation, because it possesses advantage over other sera in its constancy and its richness in the ferment in question. Since, to secure uniformity of results, it was necessary to provide a sufficiently large quantity of serum for each series of experiments, with small animals a pool had to be made of many specimens from animals killed at the same time. When guinea pigs were used, the blood was withdrawn from the heart under general anesthesia by means of a sterile test-tube provided with a sharp cannula. The blood was collected in a sterile paraffined centrifuge tube, and upon coagulation it was centrifuged to separate the serum from the clot. By this method a clear serum, absolutely free from any trace of hemolysis, may be obtained. It is important to note that for the demonstration of autodigestion of normal serum through the intervention of certain chemical substances no specimen which contains hemoglobin should be employed, since, as will be shown later, the presence of hemoglobin and stroma, whether homologous or alien, leads to the appearance of digestive products and renders the issue of the self-digestion of the serum indecisive. The experiments were carried out with fresh active serum, although it was found that the activity of the serum is not perceptibly impaired by standing at a temperature of 6°C. for many days.

The amino substances normally contained in serum were previously removed by dialysis. The serum was placed in sterile celloidin sacs and was allowed to dialyze for 5 hours at room temperature in a sterile salt solution which renewed itself from a flow from another bottle placed above the level of the dialysis vessel. The celloidin sacs were preserved in sterile distilled water with a layer of toluene

[6] Delezenne, C., and Pozerski, E., *Compt. rend. Soc. biol.*, 1903, lv, 327, cited by Jobling, J. W., and Petersen, W., *J. Exp. Med.*, 1914, xix, 460.

and before use were washed repeatedly with sterile salt solution. A layer of toluene protected the serum from bacterial interference during dialysis. The volume of the serum at the completion of dialysis was increased from one and a half times to twice its original volume. To secure a constant concentration the dialyzed serum was diluted with sterile salt solution until the volume became twice that of the original serum; that is, the dialyzed serum was made one-half of the original concentration. The dialyzed serum thus obtained, when kept in the refrigerator at 6°C., does not lose its proteolytic power for a long time, at least not for 3 or 4 weeks. We therefore kept in this way a sufficient supply of serum to complete many successive experiments with the same material.

The technique for dialysis was somewhat similar to that recommended by Abderhalden. The dialyzing thimbles used were those made by Schleicher and Schüll bearing the mark of 579A. In order to select perfect thimbles, it was necessary to test beforehand their permeability and intactness by means of solutions of silk peptone (Höchst) and egg white. Those which leaked or showed unusual porosity or retardation of dialysis were discarded as unsuitable. A serum to be tested for digestion was measured into a thimble which stood inside a sterile Jena glass wide mouthed flask. The height of the thimble and that of the flask were about the same, and the former was held upright by the edge of the latter. 15 cc. of sterile distilled water were poured into the flask outside the thimble. At the termination of dialysis, the fluid outside the thimble, representing the dialysate, was removed for determination of the amount of dialyzable proteins diffused out of the serum contained within the thimble. For this purpose the ninhydrin reaction was resorted to.[7] Since this reaction requires a temperature above 100°C. maintained for at least 1 minute, it was not easy to obtain a uniform and constant result, owing to rapid evaporation and frequent loss of the fluid incidental to the violent bubbling caused by the application of a direct flame to the test-tube containing the dialysate and ninhydrin solution. A few previous workers have attempted to eliminate errors arising from this source by using a liquid paraffin bath instead of a direct flame. The overboiling of the fluid from the test-tube placed in the paraffin oil bath at a temperature above 100°C. was greatly reduced by a specially devised stopper,[8] but we have found this device of little value, since it fails to prevent the loss of fluid by explosive escape of vapor, which forces out the fluid gathering about the narrow exit for steam.

After experiments with various devices a satisfactory result was obtained with the use of one suggested by Dr. Noguchi and illustrated in Text-fig. 1. With this

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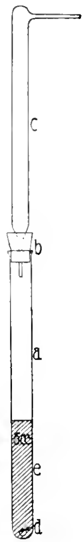
[7] The Van Slyke apparatus for the determination of amino nitrogen was also used in certain series of experiments where the amounts of the split products were sufficiently large to use this apparatus, but in ordinary experiments the amounts were too minute to permit its use.

[8] Oeller, H., and Stephan, R., Technische Neuerungen zur Dialysiermethode, *Deutsch. med. Woch.*, 1913, xxxix, 2505.

apparatus only occasionally does a small amount of the fluid escape. It consists of a hard glass test-tube (Pyrex), 1 cm. in diameter and 20 cm. in height (*a*), connected through a perforated rubber stopper (*b*) with another, somewhat narrower test-tube (*c*), the mouth of which is drawn into a long narrow neck to fit the stopper, and which has a narrow side arm near the bottom. When connected, the smaller test-tube, with narrow openings at both ends, stands in-



TEXT-FIG. 1. Apparatus used for the ninhydrin test. *a*, test-tube connected through a perforated rubber stopper (*b*) with another, narrower test-tube (*c*). In the lower test tube is a glass bead (*d*) which facilitates uniform diffusion of heat during the boiling of the mixture of dialysate and ninhydrin solution (*e*).

verted. The stopper may be lifted out with the upper tube attached and the mixture (*e*) of the dialysate (5 cc. as a rule) and ninhydrin solution (1 cc. of a 1:1,000 solution for 5 cc. of dialysate) placed in the lower test-tube, with a glass bead (*d*), which facilitates uniform diffusion of heat during the boiling. The upper portion is then tightly refitted, and the fluid is ready for boiling. The paraffin oil bath is made by filling an enameled pan with a sufficient amount of

the oil to give a depth of about 12 cm., which will cover nearly two-thirds of the height of the lower test-tube containing the fluid for heating. The bath should have width enough to hold a metal rack containing several tubes, as it is a great advantage to heat the entire series of tubes used in the experiment at the same time. It may be mentioned that the heating period is an important factor in relation to the intensity of the ninhydrin reaction. The color which manifests itself on cooling is gradually increased as the heating period is prolonged, although it was impossible to ascertain definitely, on account of the rapid evaporation of the fluid, at what rate and how long the increase proceeded. It was found, however, that the reaction at the end of 1 minute was much weaker than that of 5 minutes, and that after 10 minutes much stronger than that of 5 minutes' duration. A comparison of the intensity of the reaction was, of course, made after the volume of the fluid had been restored to the original standard by adding distilled water to the 5 cc. mark in the tube. It is easily seen, therefore, that a reaction which increases in intensity through minute errors due to inaccurate time limits would be greater during the preliminary few minutes than at the end of 5 minutes or longer. For this reason, throughout the entire experiment, instead of the 1 minute period of other investigators, we heated the fluid for exactly 5 minutes at a temperature of 150°C., or as near 150°C. as possible, the temperature being maintained by means of an oil bath in a wind-proof hood. At the end of 5 minutes the tubes were taken out of the bath and left at room temperature for 30 minutes before the reaction was read. In order to obtain a uniform and comparable result the content of each tube, which was reduced almost one-half through evaporation, was filled with distilled water up to the original volume of the dialysate; namely, 5 cc. in our experiment. The intensity of the reaction varied from a mere nuance to a distinct violet, with many intermediate grades. It was therefore necessary to prepare a standard by which different degrees of the reaction could be determined. Alanine was selected for producing the required color reaction by ninhydrin. 0.01 cc. of this substance, in 0.1 N solution, gives a distinct violet color, while 0.0025 cc. gives only a faint violet, when present in 5 cc. of distilled water. It was therefore possible to prepare a series of tubes in which color scales, based upon the gradually increasing amounts of 0.1 N alanine solution, were obtainable.

In the present study the reaction produced by 0.01 cc. of a 0.1 N solution of alanine in 5 cc. of distilled water was chosen as the standard. In an estimation of color intensity there may be two procedures. One is to have many grades of the color for comparison with a given reaction. The other, which is the one adopted in the present work, is to have one standard and to estimate the intensity of a given reaction by noting the amount of distilled water necessary to reduce the color exactly to correspond with the standard. If a given reaction requires a quadruple dilution to reach the standard, its intensity must be considered quadruple the standard; a reaction requiring triple or double dilution would be triple or double in strength.

For practical purposes, we have arbitrarily designated the reaction ++++ when the standard was attained by diluting with 3 to 3.9 cc. of water, +++ with 2 to 2.9 cc., ++ with 1 to 1.9 cc., and + with 0.9 cc. or less. Reactions weaker than this were recorded as <+ and ±, which corresponded with a mixture of 1 cc. of the standard and water up to 1 cc. and that containing more than 1 cc. of water, respectively. The reactions may be briefly summarized as follows:

++++ for a reaction requiring 3 to 3.9 cc. of water to make it correspond with the standard.

+++ for a reaction requiring 2 to 2.9 cc. of water.

++ for a reaction requiring 1 to 1.9 cc. of water.

+ for a reaction requiring 0 to 0.9 cc. of water.

<+ for a reaction corresponding with standard 1 cc. + water up to 1 cc.

± for a reaction corresponding with standard 1 cc. + water more than 1 cc.

The ninhydrin reaction with amino-acid undergoes, within a day or so, a rapid discoloration, which cannot be prevented even by preserving the tubes in a refrigerator at 6°C. A suitable substitute was sought, therefore, among various violaceous aniline dyes, and it was found that a certain high dilution of crystal violet resembles very much the ninhydrin reaction, when carefully adjusted to the standard color of the latter, and remains unaltered for a long time, provided it is kept in a dark refrigerator. The standard color solution of crystal violet was utilized for the reading of the reaction because of its stability. It should be added, however, that when subjected to further dilution, the relative color values and effect no longer run parallel.

The proteolytic activity of the serum was tested not only for the autodigestion caused by chemical reagents, but also by using as substrates some pure preparations of plant or animal proteins and various animal tissues or blood corpuscles. When animal tissues were used, they were freed from blood, boiled, and emulsified exactly as in the procedure recommended by Abderhalden. All these substrates were dialyzed in a celloidin sac before use in order to remove any dialyzable protein substances which might be contained in some of the preparations.

Occurrence of the Proteolytic Ferment in Normal Guinea Pig Serum.

To 2 cc. of the dialyzed guinea pig serum various substrates, as shown in Table I, were added and digested in thimbles for 16 hours at 37°C. The control tests done with each substrate alone gave no color reaction, whereas those done with 2 cc. of dialyzed guinea pig serum gave a reaction of only ±.

As will be seen from the table, the guinea pig serum, when incubated with some animal and plant proteins, produces dialyzable substances which show a positive ninhydrin test. Whether the serum in this case really digested the substrates, or whether the former was

TABLE I.

Effect of the Proteolytic Ferment of Normal Guinea Pig Serum on Different Substrates.

Substrate boiled.	Ninhydrin test.	Substrate boiled.	Ninhydrin test.	Substrate boiled.	Ninhydrin test.
Guinea pig liver.	+++	Rabbit placenta.	+++	Cat serum.	±
" " corpuscles.	+++	" serum.	±	" fibrin.	±
" " placenta.	+++	" fibrin.	±	Sheep corpuscles.	+++
Chicken liver.	+++	Dog corpuscles.	+++	" fibrin.	±
" corpuscles.	+++	" serum.	±	Egg white.	±
" serum.	±	" fibrin.	±	Casein (Hammersten).	+++
Rabbit liver.	+++	Cat liver.	+++	Edestin (Merck).	+++
" corpuscles.	+++	" corpuscles.	+++	Ricin (Merck).	++
Guinea pig serum.	±	Dog liver.	+++	Sheep serum.	±

brought to autodigestion only by the influence of the substrates, is not shown by this experiment. The question will be discussed in more detail below. Among the substrates tested, the serum and fibrin of various animals and egg white remained indifferent to the proteolytic ferment of serum. The presence of such a polyvalent proteolytic ferment in normal serum is already known.

Autodigestion of Normal Serum through the Action of Certain Chemicals.

Quite distinct from the proteolytic phenomenon already described is the autodigestion of normal serum brought about through the intervention of non-nitrogenous chemicals such as acetone, alcohols, and chloroform. Table II gives the results obtained when these

TABLE II.

Autodigestion of Normal Serum as a Result of Treatment with Certain Chemical Reagents.

Test No.	Dialyzed guinea pig serum.	Chemical reagents.	Digested.	Ninhydrin test.
	cc.			
1	2.0	Acetone (Kahlbaum) 0.8 cc.	In thimble at 37°C. for 16 hrs.	+++
2	2.0	Methyl alcohol (Kahlbaum) 1.0 cc.		+++
3	2.0	Chloroform (Kahlbaum), shaken, 2.0 cc.		+++
4	2.0	Salt solution 1.0 cc.		±

chemicals were added to the dialyzed guinea pig serum, and the mixture was incubated at 37°C. for 16 hours. This phenomenon seems to suggest a sort of activation of the serum ferment by these chemicals.

The object of the following experiment was to determine the optimal concentration of acetone for a given volume of serum in order to cause autodigestion. The dialyzed guinea pig serum (2 cc.) was mixed in test-tubes with 1 cc. of acetone of various concentrations. After standing for 30 minutes at room temperature, the contents of each test-tube were transferred into a thimble and digested at 37°C. for 16 hours. The dialysates outside the thimbles were tested with ninhydrin (Table III).

TABLE III.

Optimal Concentration of Acetone to Activate Serum.

Test No.	Dialyzed guinea pig serum.	Salt solution.	Acetone.	Concentration of acetone mixture.	Ninhydrin test.
	cc.	*cc.*	*cc.*	*per cent*	
1	2.0	0	1.0	$33\frac{1}{3}$	−
2	2.0	0.3	0.7	$23\frac{1}{3}$	+++
3	2.0	0.5	0.5	$16\frac{2}{3}$	+
4	2.0	0.7	0.3	10	±
5	2.0	0.8	0 2	$6\frac{2}{3}$	±
6	2.0	0.9	0.1	$3\frac{1}{3}$	±
7	2.0	1 0	0	0	±

As will be seen from the table the optimal concentration of acetone is very limited, and, according to repeated tests, it lies between 23 and 28.5 per cent. Either a lower or a higher concentration than this causes less effect, and no digestion takes place beyond a certain point. An amount of acetone which is sufficient to produce a strong turbidity or precipitation in the serum destroys the serum ferment at the same time, and there is no means of securing an active ferment by precipitating it from the serum with acetone. The same is true when the serum is precipitated with alcohol. The extreme lability of the serum ferment against acetone and alcohol presents a striking contrast to pepsin, trypsin, and other ferments, which, as is well known, withstand treatment with these reagents.

In autodigestion the serum no doubt plays the part of the ferment solution as well as of the substrate; hence, the more serum is used,

ceteris paribus, for digestion, the more split products are to be found in the dialysate. The relation of varying amounts of serum to digestion, under constant acetone concentration, was considered in the next experiment (Table IV). The result shows that under the same conditions of total liquid volume and acetone concentration the concentrated serum solution produces more dialyzable substances than the diluted one.

TABLE IV.

Relation between Various Amounts of Serum and the Degree of Autodigestion under Constant Acetone Concentration.

Test No.	Dialyzed guinea pig serum.	Salt solution.	Acetone.	Ninhydrin test.
	cc.	*cc.*	*cc.*	
1	2.0	0.2	0.8	+++
2	1.0	1.2	0.8	+
3	0.5	1.7	0.8	<+
4	0.25	1.95	0.8	±
5	0.1	2.1	0.8	–
6	0.05	2.15	0.8	–
7	2.0	1.0	0	±

Influence of Higher Temperature on the Serum Protease.

The test-tubes, each containing 2 cc. of the dialyzed guinea pig serum, were placed in the water bath regulated at 37°, 55°, and 60°C., respectively. After 30 minutes the tubes were taken out of the baths and allowed to cool at room temperature. An adequate amount of acetone or substrate (boiled chicken liver) was then added to the serum to permit detection of the presence of the ferment (Table V). The proteolytic ferment of serum, as the result shows, survives exposure to 55°C. for 30 minutes, but it is completely destroyed by heating at 60°C. for 30 minutes. The serum to which a suitable amount of acetone or a substrate had been added did not undergo autodigestion when placed in the incubator at 55°C. The optimal temperature for the action of this ferment seems to be 37°C.

TABLE V.

Resistance of the Serum Protease to Temperature.

Test No.	Dialyzed guinea pig serum.	Temperature applied.	Further treatment.	Ninhydrin test.
	cc.	°*C.*		
1	2.0	37	Acetone 0.8 cc.	+++
2	2.0		Substrate.	+++
3	2.0		Salt solution 0.8 cc.	±
4	2.0	55	Acetone 0.8 cc.	+++
5	2.0		Substrate.	+++
6	2.0		Salt solution 0.8 cc.	±
7	2.0	60	Acetone 0.8 cc.	±
8	2.0		Substrate.	±
9	2.0		Salt solution 0.8 cc.	±

Digestion Experiment in Test-Tubes without Simultaneous Dialysis.

In the preceding experiment autodigestion proceeded simultaneously with dialysis, since the serum and substrates or chemical activators were placed in a dialyzing thimble from the beginning. The question naturally arose whether or not the rate of digestion would be equally great if the mixture were put in a test-tube instead of a dialyzing thimble. There was reason to think that certain chemical activators such as acetone or alcohols would exert in test-tubes an injurious effect upon the serum ferment when added in proportions optimal for a dialyzing thimble, because in the latter a continuous reduction of the chemicals through osmosis must constitute a factor for yielding a maximum hydrolysis. In other words, the amounts of the reagents for digestion in thimbles would be too large for an optimal action of the ferment in test-tubes. This proved to be the case, as may be seen from the experiment recorded in Table VI.

Test 1 is a control test, showing the digesting power of the serum alone, without any treatment. Test 2 is another control, which demonstrates positive autodigestion caused by acetone. Test 3 shows that the serum loses its proteolytic power when mixed with acetone and kept at a temperature of 37°C. for 30 minutes. Test 4 shows that the acetonized serum which stands at room temperature for 30

minutes and then at 37°C. for 30 minutes is also inactivated. Test 5 shows that inactivation also takes place when the serum is acetonized immediately after being taken out of the water bath. Test 6 shows that a previous incubation of the serum for 30 minutes at 37°C. has no injurious effect upon the ferment action if acetone is introduced after the serum has been sufficiently cooled by standing 30 minutes after the bath.

The foregoing experiments indicate that the quantity of acetone inducing an optimal digestion of the serum in a dialyzing thimble destroys the ferment in 30 minutes when the mixture is kept at 37°C.,

TABLE VI.

Effect of Acetone upon the Serum Protease in the Test-Tube at Different Temperatures.

Test No.	Dialyzed guinea pig serum 2 cc. in test-tube.					Transferred into thimble; digested at 37°C. for 16 hrs.
	At 37°C. in water bath.	At room temperature.	Acetone.	At room temperature.	At 37°C. in water bath.	Ninhydrin test.
	min.	*min.*	*cc.*	*min.*	*min.*	
1						±
2		30	0.8	30		+++
3			0.8		30	±
4			0.8	30	30	±
5	30		0.8	30		±
6	30	30	0.8	30		+++

while no injurious effect can be detected when it is kept at room temperature for half an hour.

That the use of the dialyzing thimble is an important factor in attenuating the destructive action of acetone upon the ferment through rapid exosmosis of the reagent is shown by the presence in the dialysate of some acetone soon after dialysis began. Prevention of exosmosis of acetone from the dialyzing thimble by the addition to the outside water of acetone in exactly the same proportion as that contained in the serum within the thimble results in total inactivation of the serum protease, as will be seen from the following experiment.

In Test 7, 2 cc. of dialyzed guinea pig serum were placed in a thimble with 0.8 cc. of acetone. Instead of the usual distilled water 15 cc., a mixture of dis-

tilled water 10.7 cc., and acetone 4.3 cc., was placed outside the thimble. The concentration of acetone was then equal on both sides of the thimble. As was expected, at the end of the usual incubation period, no digestion was found to have taken place.

Unlike acetone, chloroform and tissue substrates exert no injurious action upon the ferment, even when employed in excessive quantities; hence autodigestion by means of these substances can be carried out in test-tubes.

Removal of the Activating Reagents from the Mixture with Serum.

The phenomenon of autodigestion of serum through the intervention of certain reagents, belonging chiefly to the group of so called fat solvents, arouses interest as to the causes underlying this interaction. With acetone and the simpler alcohols it was noticed that a faint opalescence appears when the reagents are mixed with serum in the optimal proportion. Whether or not this slight physical change has any relation to autodigestion is not apparent. Moreover, in the case of chloroform, which is an excellent activator, no perceptible change, except the emulsification of the serum, takes place. One might assume that autodigestion is brought about by the extraction of fatty and lipoidal substances from the serum proteins, thus enabling the serum protease to act upon the delipolyzed proteins. But ether, benzene, toluene, or petroleum ether, in spite of their delipolyzating powers, have no activating property. At all events, it seemed important to ascertain what would happen if the chemicals once mixed with the serum were extracted from the mixture. As will be shown in the following experiments, it was found that serum once acetonized or treated with other suitable chemical activators in proper proportions remains autodigestive even after the activators are completely removed. The continued presence of the activating reagents in the serum is not necessary in order to induce autodigestion.

The chemical activators can be eliminated from the mixture with serum either by (1) evaporation, (2) dialysis, or (3) treatment with other indifferent substances which free the serum from the activating chemicals.

Evaporation Method.—Evaporation by means of vacuum is preferable, because it can be done at a lower temperature and with the least risk of bacterial contamination. A temperature above 15°C. should be avoided, since, in the mixture with activators, the activity of the ferment is highly sensitive to higher temperatures.

A mixture of serum with an adequate amount of a chemical activator is put into a large sterile Petri dish, the cover replaced, and the whole placed in a desiccator, which is then exhausted by means of vacuum. As soon as the pressure drops below a certain point, the

TABLE VII.

Effect of the Removal of Acetone from the Serum Mixture by Evaporation in Vacuo.

Test No.	Dialyzed guinea pig serum.	Acetone.	Further treatment.		Digested in thimble. Dialysate. Test for acetone.	Digested in thimble. Dialysate. Ninhydrin test.
	cc.	*cc.*				
1	2.0	0	Controls. No further treatment.		–	±
2	2.0	0.8	Controls. No further treatment.		++	+++
3	2.0	0.8	Acetone evaporated to the point when bubbling ceased. Volume restored to 2 cc.		+	+++
4	2.0	0.8	Acetone evaporated to the point when bubbling ceased. Volume restored to 2 cc.	Acetone 0.8 cc.	++	+++
5	2.0	0.8	Acetone completely removed by desiccation *in vacuo*. Volume restored to 2 cc.		–	+++
6	2.0	0.8	Acetone completely removed by desiccation *in vacuo*. Volume restored to 2 cc.	Acetone 0.8 cc.	++	+++
7	2.0	0.8	Acetone completely removed by desiccation *in vacuo*. Volume restored to 2 cc.	Boiled.	–	–

contents of the dish begin to bubble. During the bubbling care must be taken to avoid loss of the liquid by overflow by regulating the speed of evaporation. The liquid ceases to bubble in a few minutes, as much of the activating reagents is already driven out of the mixture, but the odor reveals the presence of the small amount remaining. For complete removal of the reagents, evaporation must be continued until the contents of the dish are quite or nearly dried up. The residue obtained is then redissolved in sterile distilled water of a volume equal to that of the original dialyzed serum.

Acetone, chloroform, and methyl alcohol can be easily removed by this method, on account of their having a lower boiling point than the higher alcohols. But the higher series of alcohols, having a higher boiling point than that of distilled water, cannot be satisfactorily eliminated by this method.

TABLE VIII.

Effect of the Removal of Chloroform from the Serum Mixture by Evaporation in Vacuo.

<table>
<tr><th>Test No.</th><th>Dialyzed guinea pig serum.</th><th colspan="3">Further treatment.</th><th>Digested in thimble. Ninhydrin test.</th></tr>
<tr><td></td><td>cc.</td><td colspan="3"></td><td></td></tr>
<tr><td>1</td><td>2.0</td><td colspan="2">Control. No further treatment.</td><td></td><td>±</td></tr>
<tr><td>2</td><td>2.0</td><td rowspan="3">Shaken with chloroform repeatedly; stood at room temperature for 30 min.</td><td></td><td></td><td>+++</td></tr>
<tr><td>3</td><td>2.0</td><td rowspan="2">Chloroform completely evaporated. Volume restored to 2 cc.</td><td></td><td>±</td></tr>
<tr><td>4</td><td>2.0</td><td>Shaken again with chloroform.</td><td>+++</td></tr>
<tr><td>5</td><td>2.0</td><td rowspan="4">Shaken with chloroform repeatedly; stood at 6°C. for 30 min.</td><td></td><td></td><td>+++</td></tr>
<tr><td>6</td><td>2.0</td><td rowspan="3">Chloroform completely evaporated. Volume restored to 2 cc.</td><td></td><td>+++</td></tr>
<tr><td>7</td><td>2.0</td><td>Shaken again with chloroform.</td><td>+++</td></tr>
<tr><td>8</td><td>2.0</td><td>Boiled.</td><td>−</td></tr>
</table>

The behavior of the serum ferment after it has been freed from its activators by evaporation is shown in Tables VII and VIII. These tables show that acetone and chloroform can be completely removed from the mixture without any loss in the autodigestive activity of the serum, since it had already been activated by the reagents (Table VII, Test 5; Table VIII, Test 6). There is, however, a slight difference between the two reagents in their mode of action. The activating action of acetone is rather rapid, while that of chloro-

form is much slower (Table VIII, Test 3), requiring nearly 2 hours to insure an activation which will endure after the evaporation of the chemical.

Dialysis Method.—This method can be used only for the elimination of water-soluble substances, such as acetone and the lower alcohols. It is unavailable for chloroform and certain higher alcohols which are insoluble or less soluble in water. In order to utilize

TABLE IX.

Effect of the Removal of Acetone from the Serum Mixture by Evaporation and Dialysis.

<table>
<tr><th rowspan="2">Test No.</th><th rowspan="2">Dialyzed guinea pig serum.</th><th rowspan="2">Acetone.</th><th rowspan="2" colspan="3">Further treatment.</th><th colspan="2">Digested in thimble. Dialysate.</th></tr>
<tr><th>Ninhydrin test.</th><th>Test for acetone.</th></tr>
<tr><td></td><td>cc.</td><td>cc.</td><td colspan="3"></td><td></td><td></td></tr>
<tr><td>1</td><td>2.0</td><td>0</td><td colspan="2">Controls. No further treatment.</td><td></td><td>±</td><td>—</td></tr>
<tr><td>2</td><td>2.0</td><td>0.8</td><td colspan="2"></td><td></td><td>+++</td><td>++</td></tr>
<tr><td>3</td><td>2.0</td><td>0.8</td><td rowspan="3">Acetone partly evaporated immediately after having been mixed with serum.</td><td></td><td></td><td>+++</td><td>+</td></tr>
<tr><td>4</td><td>2.0</td><td>0.8</td><td rowspan="2">Residual acetone removed by dialysis for 2 hrs.</td><td></td><td>±</td><td>—</td></tr>
<tr><td>5</td><td>2.0</td><td>0.8</td><td>Acetone 0.8 cc.</td><td>+++</td><td>++</td></tr>
<tr><td>6</td><td>2.0</td><td>0.8</td><td rowspan="3">Acetone partly evaporated after standing with serum for 30 min. at room temperature.</td><td></td><td></td><td>+++</td><td>+</td></tr>
<tr><td>7</td><td>2.0</td><td>0.8</td><td rowspan="2">Residual acetone removed by dialysis for 2 hrs.</td><td></td><td>+++</td><td>—</td></tr>
<tr><td>8</td><td>2.0</td><td>0.8</td><td>Acetone 0.8 cc.</td><td>+++</td><td>++</td></tr>
</table>

celloidin membrane for dialysis it was necessary to remove, by a brief preliminary evaporation *in vacuo*, much of the reagent from the mixture, as the presence of acetone in such a concentration may affect the membrane. Complete removal of the reagent is then effected by dialysis in celloidin sacs for 2 hours in running salt solution. This combined method was used for the serum employed in Table IX.

As Test 4 shows, digestion cannot take place when the acetone is removed immediately after being mixed with the serum. If removal is begun after the mixture has already been allowed to stand at room temperature for 30 minutes, however, there is no difference in the ultimate outcome (Test 7). It is therefore advisable, in order to insure a thorough activation, to keep the mixture of serum and acetone at room temperature for at least 30 minutes before further treatment is started.

Extraction with Indifferent Fat Solvents.—As indifferent substances for the removal of chloroform or acetone from the serum by extraction, ether and petroleum ether were used, since they were found to possess neither an activating nor an injurious effect upon the serum

TABLE X.

Effect of Extraction by Means of Petroleum Ether of the Acetonized Serum.

Test No.	Kind of serum.	Further treatment.	Digested in thimble. Ninhydrin test.
1	Extracted serum 2 cc.	Alone.	+++
2		Acetone 0.8 cc.	+++
3		Emulsion of residuum 0.5 cc.	+++
4	Unextracted dialyzed guinea pig serum 2 cc. (controls).	Alone.	±
5		Acetone 0.8 cc.	+++
6		Emulsion of residuum 0.5 cc.	±

ferment. It was understood from the beginning that even by repeated and renewed extractions the acetone or alcohols cannot be completely exhausted from the serum admixtures. However, a point of interest in this mode of extraction lies in the fact that by it not only the added chemicals, but also the native fats and lipoids are removed, as is not the case in the evaporation or dialysis methods. Methyl alcohol is far less amenable to extraction from its mixture with serum, either by ether or by petroleum ether. An experiment in which this method was used follows.

4 cc. of acetone were mixed with 10 cc. of dialyzed guinea pig serum in a large centrifuge tube. After the mixture had been standing for 30 minutes at room temperature 10 cc. of petroleum ether were added to the liquid, which was then shaken energetically. The

emulsified liquid was centrifuged and the clear upper layer, consisting of petroleum ether and acetone, separated with a pipette. The extraction procedure was repeated five times and the extracted serum subsequently placed in a vacuum apparatus in order to remove the petroleum ether. The portions of petroleum ether containing fractions of acetone and representing several renewed extractions were reunited and evaporated *in vacuo*. The residue was emulsified in 1 cc. of salt solution (Table X).

As far as the experiment is concerned, the extraction of the acetone from the acetonized serum with petroleum ether makes no difference in the digesting process (Test 1). In other words, the absence of the substances of serum soluble in petroleum ether and acetone has no influence on the autodigestion of serum. It is interesting to note further that the addition of the lipoidal emulsion had neither an inhibitory action nor an accelerating influence upon the ferment activity of either the extracted (Test 3) or the unextracted (Test 6) serum. There was no antiferment in this fraction against the serum protease in question.[9]

Influence of Reactions upon the Serum Protease.

It is well known that the activity of a ferment is greatly influenced by the reaction of the medium in which it is found. In order to ascertain the optimal reaction for the serum protease, experiments were performed in which the digestion of the serum was carried out in various reactions. For this purpose amounts ranging from 0.01 to 1 cc. of a 0.1 N solution of hydrochloric acid or sodium hydroxide were added to a number of test-tubes, each containing a mixture of dialyzed guinea pig serum 2 cc., and acetone 1 cc. The total volumes of the mixtures were made uniformly 4 cc. by adding salt solution in the necessary amounts. The mixtures were allowed to stand at room temperature for 30 minutes and then were transferred

[9] The inactivity of the lipoid and fatty constituents of serum as an antiferment is attributed by Jobling and Petersen (*J. Exp. Med.*, 1914, xix, 549) to an imperfect dispersion after they are once extracted. By saponification they found them to be highly antienzymic. It seems open to discussion whether the antienzymic property of an unsaturated soap can explain the original antiferment of the serum.

to a corresponding number of dialyzing thimbles for incubation. The thimbles were placed in dialyzing flasks containing distilled water to which such quantities of acid or alkali were added as would make the reaction correspond exactly with the acidity or alkalinity of the contents of each thimble. The digestion was continued for 16 hours at 37°C. On account of the disturbing effect of acid or alkali upon the ninhydrin reaction, the acidity or alkalinity of the

TABLE XI.

Effect of Acid and Alkali on the Autodigestion of Serum.

Test No.	Dialyzed guinea pig serum.	Acetone.	Acid or alkali.	Salt solution.	Concentration of reaction in medium.*	Digested in thimble. Ninhydrin test.
	cc.	cc.	cc.	cc.		
			0.1 N hydrochloric acid.			
1	2.0	1.0	1.00	0.00	N/40 hydrochloric acid.	−
2	2.0	1.0	0.50	0.50	N/80 " "	−
3	2.0	1.0	0.25	0.75	N/160 " "	−
4	2.0	1.0	0.10	0.90	N/400 " "	±
5	2.0	1.0	0.05	0.95	N/800 " "	+++
6	2.0	1.0	0.01	0.99	N/4,000 " "	+++
7	2.0	1.0	0	1.00	0	+++
			0.1 N sodium hydroxide.			
8	2.0	1.0	0.01	0.99	N/4,000 sodium hydroxide.	+++
9	2.0	1.0	0.05	0.95	N/800 " "	++
10	2.0	1.0	0.10	0.90	N/400 " "	−
11	2.0	1.0	0.25	0.75	N/160 " "	−
12	2.0	1.0	0.50	0.50	N/80 " "	−
13	2.0	1.0	1.00	0.00	N/40 " "	−

* The figures under this heading give the resulting degrees of the reaction in the mixtures. The alkalinity of the serum itself after dialysis is weaker than $\frac{N}{1,000}$ sodium hydroxide and is therefore ignored in the calculation (Test 7).

dialysates was neutralized upon the completion of digestion (Table XI). A parallel series of experiments was carried out with alanine solution as controls.

As may be seen from Table XI, the serum protease is highly sensitive to the change in the reaction of the medium. The optimal re-

action for the ferment action is that of the dialyzed serum, or at least is within the narrow limits on each side of it, either toward acid or alkaline. Even a slight deviation in the reaction beyond these limits affects the activity of the serum ferment.

Certain Chemical Reagents as Activators of the Serum Protease.

In addition to acetone, chloroform and some alcohols were found to be ferment activators, and there may be others which behave similarly. On the other hand, ethyl ether, petroleum ether, benzene, and toluene have neither an activating nor a paralyzing action. They are indifferent towards the serum protease.

Chloroform as a ferment activator has been much discussed in preceding sections. Chloroform has as much activating power as acetone. However, the simple addition of chloroform to serum does not have much effect. The mixture must be energetically and repeatedly shaken in order to insure activation. For digestion, the emulsion of the mixture as a whole may be placed in the incubator; or one may use only the upper semitransparent layer which appears when the emulsion is allowed to stand for a few minutes at room temperature, while the greater part of the clear transparent chloroform settles at the bottom of the tube. With chloroform there is no optimal proportion to be added to the serum; the ferment is not affected at all, even when the chloroform is added in excess to the serum. That chloroform requires a longer time for activating the serum ferment than does acetone has already been noted (Table VIII).

In the following experiments some monovalent saturated alcohols and ketones were tested for their activating property.

Varying amounts of different ketones and alcohols were added to 2 cc. of the dialyzed guinea pig serum in test-tubes. Before the addition of the reagents adequate amounts of salt solution were added to the serum in order that the total volume in each test should be 3 cc. With substances which are less soluble or insoluble in serum, the mixtures were repeatedly shaken. All the tubes were allowed to stand for 30 minutes at room temperature and then were transferred into dialyzing thimbles to be placed in the incubator at 37°C. for 16 hours (Tables XII and XIII).

The ketones and alcohols behave similarly towards the serum ferment. A certain optimal concentration activates ferment, and an excess injures it. Moreover, it seems to be a rule among the reagents that the higher molecular substances of the series are generally more active than the lower ones. The optimal concentration, therefore, for activating ferment was found to be approximately 33 per cent for methyl alcohol, 23 to 27 per cent for ethyl alcohol, and 20 per cent for isopropyl alcohol. This rule seems to apply also to

TABLE XII.

Activating Power of Ketones on the Serum Protease.

Dialyzed guinea pig serum.	Salt solution.	Reagent.		Test No.	Acetone.		Test No.	Methylethyl ketone.	
		Amount.	Concentration.		Appearance of mixture.	Ninhydrin test.		Appearance of mixture.	Ninhydrin test.
cc.	*cc.*	*cc.*	*per cent*						
2 0	0	1 0	$33\frac{1}{3}$	1	Turbid.	−	7	Emulsified.	−
2 0	0 3	0 7	$23\frac{1}{3}$	2	Slight turbidity.	+++	8	"	−
2.0	0 5	0 5	$16\frac{2}{3}$	3	Clear.	+	9	"	+*
2.0	0.7	0 3	10	4	"	±	10	Slight turbidity.	+
2 0	0 8	0 2	$6\frac{2}{3}$	5	"	±	11	Clear.	±
2 0	0 9	0 1	$3\frac{1}{3}$	6	"	±	12	"	±

* The optimal concentration of methylethyl ketone for digestion +++ lies between 10 and 16.7 per cent.

ketones, though the tested substances were very few. The rule is well defined only for the lower series of substances which can be mixed with water in any proportion. In the case of the higher series, which are less soluble in water, the relation is not so constant, as will be seen from the results of tests with butyl and amyl alcohols.[10] Finally, the still higher series, such as octyl alcohols, which are not soluble in water, have no activating power for the serum ferment.

[10] The isobutyl alcohol is soluble in 10.5 parts of water at 18°C., and the isoamyl alcohol in 39 parts of water at 16.5°C.

TABLE XIII.

Activating Power of Alcohols on the Serum Protease.

Reagent.		Methyl alcohol.		Ethyl alcohol.		Isopropyl alcohol.		Isobutyl alcohol.	
Amount.	Concentration.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.
cc.	*per cent*								
1.0	$33\frac{1}{3}$	Opalescent.	+++*	Strong turbidity.	–	Strong turbidity.	–	Emulsified.	–
0.7	$23\frac{1}{3}$	"	+	Opalescent.	+++	Opalescent.	+	"	–
0.5	$16\frac{2}{3}$	"	<+	"	+	"	++†	"	–
0.3	10	Clear.	±	Clear.	±	Clear.	±	S rong turbidity.	<+
0.2	$6\frac{2}{3}$	"	±	"	±	"	±	Slight turbidity.	+++
0.1	$3\frac{1}{3}$	"	±	"	±	"	±	Clear.	±
0.07	$2\frac{1}{3}$							"	±
0.05	$1\frac{2}{3}$							"	±

Reagent.		Isoamyl alcohol.		Amyl alcohol, active		Octyl alcohol, normal.		Octyl Alcohol 2.	
Amount.	Concentration.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.	Appearance of mixture.	Ninhydrin test.
cc.	*per cent*								
1.0	$33\frac{1}{3}$	Emulsified.	<+	Emulsified.	<+	Emulsified.	±	Emulsified.	±
0.7	$23\frac{1}{3}$	"	+	"	+	"	±	"	±
0.5	$16\frac{2}{3}$	"	+	"	+++	"	±	"	±
0.3	10	"	+++	"	+++	"	±	"	±
0.2	$6\frac{2}{3}$	"	+++	"	+++	"	±	"	±
0.1	$3\frac{1}{3}$	Moderate turbidity.	+++	Slight turbidity.	+++	"	±	"	±
0.07	$2\frac{1}{3}$	Slight turbidity.	±	Clear.	+	"	±	"	±
0.05	$1\frac{2}{3}$	Clear.	±	"	±	"	±	"	±
0.03	1	"	±	"	±	"	±	"	±
0.02	$\frac{2}{3}$	"	±	"	±	"	±	"	±
0.01	$\frac{1}{3}$	"	±	"	±	"	±	"	±

* The methyl alcohol, when added in a 1.5 cc. amount to 2 cc. of dialyzed serum, destroys the ferment.

† The concentration of isopropyl alcohol giving the maximal digestion is about 20 per cent.

SUMMARY AND CONCLUSIONS.

1. By means of certain chemical reagents, normal guinea pig serum can be brought to autodigestion without the presence of any foreign substrate. There exists in normal sera a highly characteristic protease.

2. The serum ferment survives heating at 55°C. for 30 minutes, but is completely inactivated at 60°C. for the same length of time.

3. The autodigestion of serum requires a temperature of about 37°C., and no noticeable digestion takes place at a temperature of 16°C. or lower.

4. Autodigestion of the serum may be brought about by chloroform and various saturated monovalent ketones and alcohols of the lower series.

5. The ketones and alcohols have a certain narrow limit of concentration for activating serum, beyond which the ferment is destroyed, even at room temperature.

6. The ketones and alcohols in concentrations regulated to activate serum at room temperature destroy the ferment when allowed to act on serum at 37°C. for 30 minutes. The elimination of the concentrated reagents from serum by evaporation or dialysis protects the ferment from their destructive action.

7. A certain length of time is required for the chemical activators to complete their action. In this respect chloroform is much slower than acetone.

8. The chemical activators may be removed from the activated serum by means of vacuum, dialysis, or extraction with certain indifferent chemicals without causing a return of the serum to its original non-autolytic state. Once activated by these reagents, the serum remains in the activated state, in spite of the removal of the activators.

9. The ferment is highly sensitive to the reaction of the medium, being readily inactivated when the reaction exceeds a certain narrow limit towards acid or alkaline. The optimal digestion is obtained with a faintly alkaline or neutral reaction.

This work was done in the laboratory of Dr. Hideyo Noguchi, under his direction.

THE AUTODIGESTION OF NORMAL SERUM THROUGH THE ACTION OF CERTAIN CHEMICAL AGENTS. II.

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It has been shown that normal serum contains a characteristic protease whose activity is revealed through the action of certain chemical activators.[1] The seroprotease shows a thermal resistance similar to that of certain proteolytic enzymes, but is peculiar in its ready destruction by the action of acetone or alcohol, to which other ferments manifest a high degree of resistance.

In the present paper we have considered the relation which this ferment bears to the various fatty and lipoidal substances and also the existence of an antiferment in serum and its relation to the seroprotease. The material and method of study have been described in the previous paper.[1]

Relation of Neutral Fats, Fatty Acids, and Lipoids to the Serum Protease.

Since all the reagents, the activating effects of which have been discussed in the previous paper, belong to the group of so called fat solvents, it is not out of place to consider what part the fat or lipoid bodies may play in the autodigestion of serum caused by these reagents. The experiments were carried out partly by adding excessive amounts of fats or lipoids to the digesting mixtures, and partly by removing the native fats and lipoids from the serum by the use of fat solvents.

Several preparations of neutral fats and lipoid bodies were dissolved in acetone in high concentration, with the exception of lecithin, which, on account of its insolubility in acetone, was dissolved in methyl alcohol. Each substance was added to 2 cc. of the dialyzed

[1] Yamakawa, S., The autodigestion of normal serum through the action of certain chemical agents. I, *J. Exp. Med.*, 1918, xxvii, 689.

guinea pig serum in two different concentrations. Some of the solutions precipitated particles of the substance when mixed with the serum and formed a layer near the surface. After standing for 30 minutes at room temperature, the contents of each test-tube were transferred into a dialyzing thimble and incubated at 37°C. for 16 hours (Table I).

That cholesterol, lecithin, and the neutral fats such as triolein and tripalmitin, even when they are added in excess to the serum, are indifferent to the process of autodigestion is proved by these experiments. The weakness of digestion in cases where fatty acids are added to the serum may be explained in various ways. As was stated in the previous paper,[1] the serum ferment is extremely sensitive to an acid reaction and is undoubtedly influenced by the fatty acids. The inhibiting power of the oleic acid was found to be much stronger than that of the palmitic acid (Tests 13, 14, 17, and 18), when they are allowed to act upon the serum ferment in equal concentration. It is not improbable that the weakness of the latter is chiefly due to its inferior solubility in a medium containing much water and to its higher melting point.

The phenomenon might be explained in another way; namely, by a specific inhibiting power of an unsaturated fatty acid such as oleic against the serum ferment. Jobling and Petersen[2] found that the unsaturated fatty acids in serum act as antitrypsin, and that they can be removed by extraction with ether or chloroform. But their results with trypsin do not find an analogy with the serum protease. As has been said, acetone or chloroform can impart their activating power to the serum ferment, and they do so without eliminating any of the native elements from it; a subsequent removal of the reagents from the activated serum does not restore the original resistance to autodigestion. Moreover, ether, toluene, benzene, and petroleum ether do not act as activators for the serum protease. The following experiment was undertaken in order to determine the effect of complete removal of the fats, fatty acids, and lipoids from the serum upon the phenomenon of autodigestion.

[2] Jobling, J. W., and Petersen, W., The nature of serum antitrypsin, *J. Exp. Med.*, 1914, xix, 459.

TABLE I.

Effect of Fatty Substances on the Autodigestion of Serum.

Test No.	Acetone solution of fats added to 2 cc. of dialyzed guinea pig serum.		Acetone.	Acid reaction. In thimble	Acid reaction. Of dialysate.	Ninhydrin test.
		cc.	*cc.*			
1	10 per cent oleic acid.	0.8	0	+	–	<+
2		0.08	0.72	+	–	++
3	10 per cent triolein.	0.8	0	–	–	+++
4		0.08	0.72	–	–	+++
5	0.3 per cent palmitic acid.	0.8	0	<+	–	++
6		0.08	.72	<+	–	+++
7	0.3 per cent tripalmitin.	0.8	0	–	–	+++
8		0.08	0.72	–	–	+++
9	Cholesterol saturated.	0.8	0	–	–	+++
10		0.08	0.72	–	–	+++
11	Guinea pig serum alone (controls).		0.8			+++
12			0			±

Test No.	Chloroform solution of fats added to 2 cc. of dialyzed guinea pig serum.		Chloroform.	Acid reaction. In thimble.	Acid reaction. Of dialysate.	Ninhydrin test.
		cc.	*cc.*			
13	10 per cent oleic acid.	1.0	0	+	–	<+
14		0.1	0.9	+	–	++
15	10 per cent triolein.	1.0	0	–	–	+++
16		0.1	0.9	–	–	+++
17	10 per cent palmitic acid.	1.0	0	+	–	++
18		0.1	0.9	+	–	++
19	10 per cent tripalmitin.	1.0	0	–	–	+++
20		0.1	0.9	–	–	+++
21	Guinea pig serum alone (controls).		1.0			+++
22			0			±

TABLE I—*Concluded.*

Test No.	Dialyzed guinea pig serum.	1 per cent ovo-lecithin emulsion in salt solution	Salt solution.	Methyl alcohol.	Acid reaction.		Ninhydrin test.
					In thimble.	Of dialysate.	
	cc.	*cc.*	*cc.*	*cc.*			
23	2.0	1.0		1.5	−	−	+++
24	2.0	0.1	0.9	1.5	−	−	+++
25	2.0	1.0	0	0	−	−	±
26	2.0	0	1.0	1.5	−	−	+++

10 cc. of guinea pig serum were completely dried in the desiccator by means of vacuum. The residue was ground into powder, placed in a flask, and treated with 100 cc. of absolute ether. The flask was kept for 48 hours in the refrigerator, repeatedly shaken at intervals, and the solvent three times renewed. At the expiration of this period the ether was decanted, the precipitate was washed with another 100 cc. of ether, and the trace of the solvent was removed *in vacuo*. The dried powder was then dissolved in 10 cc. of sterile distilled water and dialyzed in salt solution to remove the dialyzable substances. After dialysis the serum was diluted to 20 cc. with salt solution and used for the tests (Table II).

TABLE II.

Autodigestion of the Serum Delipolyzed with Ether.

Test No.	Extracted guinea pig serum.	Further treatment.	Ninhydrin test.
	cc.		
1	2.0	No further treatment.	±
2	2.0	Acetone 0.8 cc. added.	+++
3	2.0	Shaken with 1 cc. of chloroform.	+++
4	2.0	Methyl alcohol 1 cc. added.	+++
5	2.0	Substrate (chicken liver) added.	+++

The experiment shows that the extraction of fatty substances from the dried serum with ether causes no change with regard to the phenomenon of autodigestion of the serum.

The Inhibitory Substance in Native Serum against the Serum Protease.

It is generally known that human or animal serum has an inhibitory effect upon various proteolytic ferments, such as pepsin, trypsin, leukoprotease, and autolytic ferment. The results of the investiga-

tions on the influence of serum on the serum protease will be described here.

The investigation divided itself into two parts: (1) the digestion of heterologous substrate by the guinea pig serum ferment, and (2) the autodigestion of serum caused by the chemical reagents already mentioned. In the latter case particular care was taken to remove the chemical reagents completely from the treated serum before the sample of native serum which was to be tested for its inhibitory power was added, because, should any trace of the activators still be present, it would lead to an activation of the serum thus introduced. Acetone was used throughout the experiment because of the ease with which it can be completely removed from the serum mixtures. The dialyzed serum, acetonized and then deacetonized, will be designated, for the sake of brevity, as "activated serum."

The result shown in Table III indicates that the larger the amount of the dialyzed guinea pig serum added, the greater is the digestion of the substrate. On the other hand, the addition of a dialyzed horse serum caused neither increase nor decrease of digestion by guinea pig serum (Table IV). The horse serum itself was inactive.

The result of the autodigestion test with activated serum distinctly shows the presence of an inhibitory substance in a dialyzed but otherwise unmodified serum (Table V). The contradictory results in both cases will be discussed later.

The serum antienzymes directed against various proteolytic ferments disappear from the serum when the latter is heated to a certain temperature. The two following experiments were undertaken to determine the thermal resistance of the antiseroprotease.

1 cc. of dialyzed guinea pig serum was heated in the water bath at different temperatures for varying periods of time. The heated sera, after having been cooled, were added to 2 cc. of the activated serum in tubes, allowed to stand at room temperature for 30 minutes, and transferred as usual into thimbles for incubation and dialysis (Table VI).

According to this experiment, the inhibitory substance in unmodified or native serum withstands heating at 55°C. for 30 minutes, whereas it is destroyed by exposure at 60°C. for the same period. The thermal resistance of the antiseroprotease coincides with that

TABLE III.

Digestion of a Substrate with Guinea Pig Serum in Increasing Quantities

Dialyzed guinea pig serum.	Salt solution.	Digested in thimble with chicken liver. Ninhydrin test.	Digested in thimble without substrate Ninhydrin test.
cc.	*cc.*		
2.0	2.0	+++	±
3.0	1.0	++++	±
4.0	0	++++	<+

TABLE IV.

Effect of a Heterologous Serum on the Digestion of a Substrate by Guinea Pig Serum.

Dialyzed guinea pig serum.	Dialyzed horse serum.	Salt solution.	Digested in thimble with chicken liver. Ninhydrin test.	Digested in thimble without substrate. Ninhydrin test.
cc.	*cc.*	*cc.*		
2.0	0	2.0	+++	±
2.0	1.0	1.0	+++	±
2.0	2.0	0	+++	±
0	2.0	2.0	±	—

TABLE V.

Inhibitory Power of the Homologous Serum against the Autodigestion of an Activated Guinea Pig Serum.

Test No.	Activated guinea pig serum.*	Homologous guinea pig serum.*	Salt solution.	Digested in thimble. Ninhydrin test.
	cc.	*cc.*	*cc.*	
1	2.0	2.0	0	<+
2	2.0	1.0	1.0	<+
3	2.0	0.5	1.5	<+
4	2.0	0.25	1.75	++
5	2.0	0.1	1.9	+++
6	2.0	0.05	1.95	+++
7	2.0	0	2.0	+++
8	0	2.0	2.0	±

* Both sera were previously dialyzed, and the mixture of both had been allowed to stand for 30 minutes at room temperature before being placed in the incubator at 37° C.

TABLE VI.

Inactivation of the Antiseroprotease of Guinea Pig Serum by Heating.

Test No.	Activated guinea pig serum.	Guinea pig serum 1 cc. exposed to various temperatures.	Ninhydrin test.
	cc.		
1	2.0	Not heated. Clear.	<+
2	2.0	30 min. at 55°C. Clear.	<+
3	2.0	30 " " 60° " Slightly turbid.	+++
4	2.0	30 " " 65° " Opalescent.	+++
5	2.0	30 " " 70° " "	+++
6	2.0	5 " " 100° " Coagulated.	+++
7	2.0	Salt solution 1 cc.	+++
8	Salt solution 2 cc.	Not heated. Clear.	±

TABLE VII.

Thermal Resistance of the Protease and Its Antisubstance in Serum.

Tests for protease.				Tests for antisubstance.				Heated serum alone.		
Test No.	Native guinea pig serum 1 cc. heated at 55°C. for.	Acetone added.	Ninhydrin test.	Test No.	Native guinea pig serum 2 cc. heated at 55° C. for.	Activated guinea pig serum added	Ninhydrin test.	Test No.	Native guinea pig serum 2 cc. heated at 55° C. for.	Ninhydrin test.
	min.	*cc.*			*min.*	*cc.*			*min.*	
1	30	0.8	+++	6	30	2.0	<+	12	30	±
2	60	0.8	+	7	60	2.0	++	13	60	±
3	120	0.8	±	8	120	2.0	+++	14	120	±
4	240	0.8	—	9	240	2.0	+++	15	240	—
5	Guinea pig serum not heated, 2 cc.	0.8	+++	10	Guinea pig serum not heated, 1 cc.	2.0	<+	16	Guinea pig serum not heated, 2 cc.	±
				11	Salt solution 1 cc.	2.0	+++			

of the serum protease itself.[3] This fact was proved again in the next experiment.

The unmodified or native guinea pig serum, 1 cc., was exposed to a temperature of 55°C. for various periods of time. Each heated serum was mixed

[3] Yamakawa,[1] Table V.

with acetone or activated serum respectively, to be tested for its proteolytic and antiproteolytic power. After standing for 30 minutes at room temperature the mixtures were transferred into thimbles and placed in the incubator (Table VII).

Exposed to a temperature of 55°C., both the ferment and the antisubstance remain unimpaired for 30 minutes, but their activity gradually diminishes after a longer time, finally disappearing after 2 hours. A dissociation of the ferment from its antisubstance through heating was found to be impossible.

Effect of the Adsorbing Substances on the Serum.

Certain inorganic substances, which had been previously sterilized by heating, were put into the dialyzed guinea pig serum in a proportion of 5 gm. to 10 cc. The mixtures were allowed to stand at room

TABLE VIII.

Digesting Power of the Serum Treated with Adsorbents.

Test No.	Kind and amount of guinea pig serum.	Further treatment.	Ninhydrin test.
1	Serum treated with kaolin 2 cc.	No further treatment.	–
2		Acetone 0.8 cc. added.	–
3		Substrate added.	–
4	Serum treated with charcoal 2 cc.	No further treatment.	–
5		Acetone 0.8 cc. added.	±
6		Substrate added.	–
7	Serum treated with talc 2 cc.	No further treatment.	–
8		Acetone 0.8 cc. added.	±
9		Substrate added.	–
10	Serum treated with silicious marl 2 cc.	No further treatment.	–
11		Acetone 0.8 cc. added.	±
12		Substrate added.	–
13	Serum treated with barium sulfate 2 cc.	No further treatment.	–
14		Acetone 0.8 cc. added.	±
15		Substrate added.	–
16	Untreated serum 2 cc. (controls).	No further treatment.	±
17		Acetone 0.8 cc. added.	+++
18		Substrate added.	+++

temperature for an hour, with repeated shakings, and then centrifuged. The clear supernatant fluids were used for the experiment (Table VIII).

As may be seen from Tables VIII and IX, the proteolytic ferment can be easily removed from serum by adsorbents, but the antisubstance, on the other hand, still remains in the treated serum.

TABLE IX.

Antienzymic Action of the Serum Treated with Adsorbents.

Test No	Kind and amount of guinea pig serum.		Activated serum added.	Acetone added.	Ninhydrin test.
		cc.	*cc.*	*cc.*	
1	Guinea pig serum treated with kaolin.	2.0	0	0	–
2		2.0	0	0.8	–
3		1.0	2.0	0	<+
4	Guinea pig serum treated with talc.	2.0	0	0	–
5		2.0	0	0.8	±
6		1.0	2.0	0	<+
7	Salt solution.	1.0	2.0	0	+++
8	Guinea pig serum.	1.0	2.0	0	<+

Occurrence of the Proteolytic Ferment and Its Antisubstance in the Sera of Different Animals.

It would surely have been an advantage if we could have found larger animals which would furnish us with a serum as rich in the

TABLE X.

The Proteolytic Ferment and Its Antisubstance in the Sera of Different Animals.

Kind of serum.	No. of tested specimens.	Dialyzed serum alone 2 cc.	Dialyzed serum 2 cc. + acetone 0.8 cc.	Dialyzed serum 1 cc. + activated guinea pig serum 2 cc.
Human serum	2	+	<+	<+
Dog "	5	±	<+	<+
Cat "	2	±	<+	<+
Rabbit "	8	±	<+	<+
Horse "	2	–	<+	<+
Guinea pig serum	Over 100	±	+++	<+

serum protease as that of the guinea pig. The results of examinations of various animal sera, however, showed that the guinea pig is the only animal whose serum is exceedingly rich in the proteolytic ferment. On the other hand, the sera of other animals, while poor in their content of protease, contain a considerable amount of the antisubstance capable of counteracting the action of the autolytic ferment of guinea pig serum., The result of the digestion tests with the sera of different animals is shown in Table X.

Mode of Digestive Action of the Serum Ferment.

It has been stated in a previous paragraph that the proteolytic ferment of serum, when it is incubated with substrate, can produce the dialyzable substances despite the presence of native serum, while in the autodigestion of activated serum, the ferment action is inhibited by the addition of native serum. There seems to be a certain difference in the mode of action in the two instances.

The explanation of the autodigestion of the activated serum may probably be sought in the destruction or paralysis of the antienzymic substance through the treatment. Reagents such as certain ketones and alcohols, when their optimal concentration for activation is reached, may destroy the antienzyme, but not the enzyme, thus enabling the latter to exert its full activity upon the serum proteins. The concentration of reagent which dissociates the ferment from its antisubstance lies between narrow limits, and when it exceeds the upper limit, the ferment itself is also destroyed.

In autodigestion the protein in the treated serum must serve as substrate, because there is nothing else present to be hydrolyzed. But what is the origin, then, of the dialyzable substance produced when the serum is incubated with various tissue substrates? There are two possibilities for the source of the protein derivatives: first, the substrates may be directly digested by the serum ferment; second, it may be assumed that the homologous tissues are not really digested, but that they act only as an adsorbing agent which removes the antienzyme and leaves the freed autolytic ferment to digest its own serum protein. The latter explanation was advanced by Bron-

fenbrenner[4] in the Abderhalden reaction, in which pregnant human serum, when incubated with placenta tissue, gives a positive ninhydrin test. He states that pregnant serum is able to show autodigestion in the incubator when allowed to remain in contact with

TABLE XI.

Antienzymic Action of Normal Serum after Treatment with Substrate at 0.5°C.

<table>
<tr><th>Test No.</th><th>Dialyzed guinea pig serum.</th><th colspan="3">Further treatment.</th><th>Digested in thimble. Ninhydrin test.</th></tr>
<tr><td></td><td>cc.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>1</td><td>2.0</td><td rowspan="5">Substrate added. Tubes left at 0.5° C. for 16 hrs.</td><td rowspan="3">Centrifuged. Substrate removed. Supernatant fluid alone used for tests.</td><td></td><td>±</td></tr>
<tr><td>2</td><td>2.0</td><td>Boiled.</td><td>−</td></tr>
<tr><td>3</td><td>2.0</td><td>Acetone 0.8 cc.</td><td>+++</td></tr>
<tr><td>4</td><td>1.0</td><td></td><td>Activated guinea pig serum 2 cc.</td><td><+</td></tr>
<tr><td>5</td><td>2.0</td><td>Substrate in situ.</td><td></td><td>+++</td></tr>
<tr><td colspan="6">Control tests.</td></tr>
<tr><td>6</td><td>2.0</td><td colspan="3">Without any treatment.</td><td>±</td></tr>
<tr><td>7</td><td>2.0</td><td colspan="3">Acetone 0.8 cc. added.</td><td>+++</td></tr>
<tr><td>8</td><td>2.0</td><td colspan="3">Substrate added.</td><td>+++</td></tr>
<tr><td>9</td><td>1.0</td><td colspan="3">Activated guinea pig serum 2 cc. added.</td><td><+</td></tr>
<tr><td>10</td><td>Salt solution 1 cc.</td><td colspan="3">Activated guinea pig serum 2 cc. added.</td><td>+++</td></tr>
</table>

placenta tissue for 16 hours on ice and then separated from the substrate. He ascribes the phenomenon to adsorption of the antienzymic substance by the substrate impregnated with a specific antibody contained in the serum of a pregnant subject.

The next experiment was undertaken to determine whether this

[4] Bronfenbrenner, J., On the present status of the Abderhalden reaction, *J. Lab. and Clin. Med.*, 1915–16, i, 79.

mode of interpretation was applicable in our case, in which a heterologous, non-specific substrate is treated with normal serum (Table XI).

Tests 1 and 4 show that normal guinea pig serum, when it is kept on ice with substrate, is neither activated nor deprived of its antienzymic substance. In other words, the normal serum is indifferent to the treatment, contrary to the result which Bronfenbrenner reported to have obtained with human pregnant serum and placenta tissue. But this result does not exclude the possibility of the adsorption of the antisubstance by ordinary substrates in the incubator at a temperature of 37°C.

To determine the fate of the antienzymic substance in serum, after digestion, the serum was treated according to four methods, as follows:

Serum A.—8 cc. of the dialyzed guinea pig serum were kept in the incubator with substrate in four thimbles for 16 hours, 2 cc. being placed in each thimble. At the expiration of this time, when the dialysate showed a ninhydrin reaction of +++, the serum in the thimbles was separated from the substrate layer and every trace of the latter removed by means of centrifugation. The serum was then dialyzed in a celloidin sac against salt solution to eliminate the split products of protein contained in it.

Serum B.—8 cc. of the activated guinea pig serum were kept in thimbles in the incubator for 16 hours. The ninhydrin test of the dialysate showed a reaction of +++. The sera in the thimbles were reunited and dialyzed as mentioned above.

Serum C (Control 1).—The dialyzed guinea pig serum, 8 cc., without any substrate and without being activated, was treated in the same way as the other two sets of serum; *i.e.*, incubated in thimbles and afterwards dialyzed.

Serum D (Control 2).—The dialyzed guinea pig serum without any preliminary treatment.

These sera were further treated as shown in Table XII and digested in thimbles at 37°C. for 16 hours. The dialysates were tested as usual.

The result of this experiment indicates that the serum, the proteolytic power of which has already been exhausted by treatment with substrate, still contains its antienzymic substance (Serum A, Test 4), while the latter is no longer found in the activated serum after digestion (Serum B, Test 4). There is no doubt that in the former case the digestion can take place in spite of the presence of

the antienzymic substance. We have no more reason in this instance to assume the occurrence of an indirect digestion of serum protein, due to the absorption of the antienzymic substance through the substrate, because the substrate does not absorb the antienzyme under the experimental conditions here recorded. It seems justifiable, therefore, to conclude that the serum ferment directly digests the protein of the heterologous substrate, while in the case of the activated serum, the ferment splits its own serum protein after the antienzymic substance has been removed by the treatment with ketones or alcohols.

TABLE XII.

Fate of the Antienzyme Substance in Serum after Digestion.

Test No.	Amount of serum.*	Further treatment.	Serum A	Serum B	Serum C	Serum D
	cc.					
1	2.0	No further treatment.	—	—	—	±
2	2.0	Acetone 0.8 cc.	—	±	++	+++
3	2.0	Substrate.	—	+	++	+++
4	1.0	Activated guinea pig serum 2 cc.	<+	+++	<+	<+
5	1.0	Dialyzed guinea pig serum 2 cc. + substrate.	+++	+++	+++	+++

* The volume of the serum was increased after secondary dialysis by about one-fourth.

SUMMARY AND CONCLUSIONS.

1. The neutral fats, fatty acids, and lipoid bodies of serum seem to play no part in autodigestion. Neither the addition of fats or lipoids in excess to the serum, nor their removal by extraction with ether influences the phenomenon of autodigestion.

2. There is present in native serum an antienzymic substance which is closely related to the autolytic ferment of serum.

3. The antiseroprotease of normal serum has almost the same thermal resistance as the seroprotease; that is, it survives heating at 55°C. for 30 minutes but is completely inactivated at 60°C. for the same length of time.

4. The ferment can be removed from the serum by means of inorganic adsorbents, but the antienzymic substance remains in the treated serum.

5. The autolytic power of the sera of man and other animals is much weaker than that of guinea pig serum, but they contain as much as does the latter of the antisubstance which inhibits the digestion of the activated guinea pig serum.

6. The autodigestion of the activated serum is due to the splitting of the serum protein by the proteolytic ferment of the same serum and is brought about by the destruction of the antienzymic substance by the chemical reagents. On the other hand, the digestion products in a mixture of a foreign substrate and guinea pig serum are derived from the direct digestion of the substrate by the serum ferment. This digestion takes place in spite of the presence of the antiseroprotease. The serum separated from the substrate can no longer produce a split product, but is as actively antienzymic as the original serum and undergoes autodigestion only when treated with acetone or other chemical activators.

This work was done in the laboratory of Dr. Hideyo Noguchi, under his direction.

ANTIBODY PRODUCTION AFTER PARTIAL ADRENALECTOMY IN GUINEA PIGS.

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Aside from the well established action of products of adrenal secretion upon sympathetic nerve endings and their consequent effect upon striated and smooth musculature, there has been assigned to the adrenal glands a detoxifying action upon endogenous and exogenous poisons which would class them among the specific defensive mechanisms of the body. Studies of this action have been limited largely to observations and experiments to show the effects of acute and chronic intoxications upon the adrenal glands, the symptomatology of adrenal hyperplasia or degeneration, the effect of partial or complete extirpation upon the toxicity of various substances, or the neutralization of toxins by adrenal extracts in the test-tube or the living animal. The literature is confused and contradictory, and much of the evidence brought forward is speculative and indirect, due in part to the use of the death or survival of the animal as sole indicator, and the consequent introduction of many unknown factors into the experimental equation, without critical analysis of the mechanism involved.

Only a few experiments have come to light which bear directly upon a possible relation between the adrenal glands and the recognized immunity factors of the defensive mechanism, and these reported findings are almost entirely of a negative significance. Thus Josué and Paillard[1] found that injections of adrenalin or of adrenal extracts into rabbits had no effect on the opsonic properties of the blood. Hektoen and Curtis[2] report that "Adrenalectomy in normal dogs, and in dogs at the height of the antibody curve after the injection of rat blood, did not cause any fall in the antibody content of the blood serum, as determined by hourly

[1] Josué, O., and Paillard, H., Influence de l'adrénalin sur le pouvoir opsonique (Première note), *Compt. rend. Soc. biol.*, 1910, lxviii, 657.

[2] Hektoen, L., and Curtis, A. R., The effect on antibody production of the removal of various organs, *J. Infect. Dis.*, 1915, xvii, 409.

observations after the operation and until death." Gay and Rusk[3] in presenting studies on antibody formation, in which the literature is reviewed, make no mention of the adrenals as possible sources of immune substances, and all the positive evidence reported in regard to the origin of immune bodies points to other organs, notably the lymph glands, bone marrow, and spleen, as the tissues probably concerned. A solitary communication by Cattoretti[4] states that the addition of pancreas extract to the blood of adrenalectomized rats gave a marked lowering of the surface tension (miostagmin reaction) compared with the normal lowering produced by the extract.

There is no confirmed evidence that the adrenal glands play an active part in antibody formation, or in the known immunity reactions of defense against bacterial invasion. On the other hand, the few experiments which have been made to study such a relation have given negative results.

As a part of a more general investigation of the possible relation of glands of internal secretion to immunity processes we have made experiments on the effect of partial adrenalectomy upon antibody formation in guinea pigs. For this purpose the animals were subjected to operation before or after immunization with a typhoid vaccine or with washed red blood corpuscles of the hen, and their typhoid agglutinins or hemolysins and hemagglutinins titered at intervals during the course of antibody production.

Healthy adult guinea pigs, usually males, weighing from 300 to 400 gm., served as the experimental animal. For the purposes of the experiments it was necessary that the animals should survive during the interval required for subsequent active immunization or for the change in antibodies already in circulation, in the event that adrenalectomy modified the response. Consequently, complete removal of the glands was interdicted, since guinea pigs are practically without accessory adrenal tissue and almost invariably die within a few hours of a total extirpation.[5]

Operations for the partial removal of the adrenals were performed

[3] Gay, F. P., and Rusk, G. Y., Studies on the locus of antibody formation, *Tr. XVth Internat. Cong. Hyg. and Demog., Washington (1912)*, 1913, ii, 328.

[4] Cattoretti, F., Ueber die Meiostagminreaktion bei den weissen Ratten nach Extirpation der beiden Nebennieren, *Wien. klin. Woch.*, 1911, xxiv, 637.

[5] Lucien, M., and Parisot, J.-V.-J., Glandes surrénales et organes chromaffines, Paris, 1913, 108.

with careful aseptic technique, and the endeavor to reduce shock to the minimum by prompt hemostasis, the use of a warm pad during and after operation, machine-controlled anesthesia with constant ether concentration, and saline injections when indicated. We deemed it essential carefully to avoid complications due to infection or to injury to other organs in the course of the operation.

Operation.—Oblique incisions on both sides, separating the last two ribs, and extending to the edge of the sacrospinal muscles gave a clear exposure of the glands with the least disturbance of the abdominal contents. The adrenals were dissected free to the hilum, caught in a delicate curved mosquito clamp at the base or through their substance, and cut free distal to the clamp. Only slight bleeding resulted if the clamp was left in place a short time. The glands or portions of glands removed were weighed to estimate the amount taken and sectioned for comparison post mortem with the segment left in place.

It was soon found that the guinea pig could stand the loss of the whole of one gland and from one-half to three-fourths of the other. If too much tissue was taken the animal died in from a few hours to several days, after showing characteristic symptoms. A marked fall in temperature (to 28°C. in one instance), extreme prostration, gasping respiration, intermittent clonic convulsions, and, in males, the extrusion of semen immediately preceded death. These findings are in accord with former reports.

On the other hand, surviving guinea pigs recovered quickly from the operation and remained well for months, sometimes losing at first 50 to 100 gm. in weight, which was often recovered later. Some of the animals died during the course of the experiments, however, from hemopericardium after cardiac puncture or from an intercurrent lung epizootic prevalent among the stock.

Technique of the Serum Reactions.—The strain of *B. typhosus* chosen was a stock culture known as "Sen," recovered by Dr. Bull from an ampule of Besredka's sensitized vaccine. It had been on artificial media for several years and combined ready agglutinability with a high toxicity for guinea pigs, often killing them in the usual immunizing doses. For this reason and to obtain exact dosage, a vaccine was prepared by suspending 24 hour growths from Blake bottles in saline solution, killing the bacilli with chloroform, disrupting them by repeated freezing and thawing, dehydrating *in vacuo* over sulfuric acid, and powdering in a mortar. Weighed quantities were resuspended in normal saline solution, extracted by shaking for several hours, and measured doses corresponding to 0.5

to 2 mg. of the powder were injected intraperitoneally at 3 to 4 day intervals for three doses. Initial agglutinin titers ranged from 1: 640 to 1: 5,120 on the 7th to 10th day after the final injection. Ten of twenty-four guinea pigs gave an initial titer of 1: 1,280. In order to simplify the experiments only the typhoid agglutinins were followed. Casual tests showed that this method of immunization, while simple and rapid, did not produce precipitins or complement-fixing bodies in concentrations suitable for investigation.

Other guinea pigs were immunized with red blood corpuscles of barred Plymouth Rock chickens. Following Coca's[6] schedule three intraperitoneal doses of 0.5 to 1.5 cc. of washed corpuscles, made up to the original blood volume, were injected at 4 day intervals and the guinea pigs were first bled a week later. The first hemagglutinin titers ranged from 1: 160 to 1: 1,280, hemolysins appearing in dilutions five times as great, taking into account the dilutions involved in the hemolytic system. For the agglutination tests one drop of a suspension of *B. typhosus*, Sen, or of washed hen corpuscles (10 per cent of the original blood volume), was added to a 1 cc. volume of successive dilutions of the fresh, inactivated guinea pig serum. The tubes were read first after 2 hours at 37°C. and 2 hours at room temperature. A confirmatory reading was taken after the tubes had stood in the ice box over night. To test hemolysins, 0.25 cc. volumes of inactivated experimental serum, fresh guinea pig complement 1: 10, and 5 per cent hen corpuscle suspension were made up with 0.5 cc. of saline solution, following the usual technique of the Wassermann reaction. These tubes were incubated $1\frac{1}{2}$ hours, being shaken at the half hour and hour, and read immediately, and after standing in the ice box over night. All the tests were performed with the usual controls.

With these methods several series of experiments were performed. Usually three guinea pigs formed the experimental unit. In some instances two were partially adrenalectomized, the other serving as a normal control. In other cases two animals served as controls, one normal, the other after an operation similar to double adrenalectomy except for the removal of the glands. The control operated animals showed that the operation itself had no influence on antibody formation.

Series I.

In some of the experiments in this series the guinea pigs were first immunized with *Bacillus typhosus* vaccine and their agglutinin titers recorded on the 7th day after the third injection. Within a few days

[6] Coca, A. F., A rapid and efficient method of producing hemolytic amboceptor against sheep corpuscles, *J. Infect. Dis.*, 1915, xvii, 361.

(8 to 11 days after immunization) the adrenalectomies or control operations were performed and the agglutinin titers followed in 2 cc. samples of blood obtained by cardiac puncture at intervals during the succeeding weeks and months. In other experiments the operations preceded immunization, which was begun from 1 to 52 days later. In a few instances one gland or a part of a gland was removed, the

TABLE I.

Differences in Agglutinin Titer of Various Sera on Reexamination after an Interval of Time.

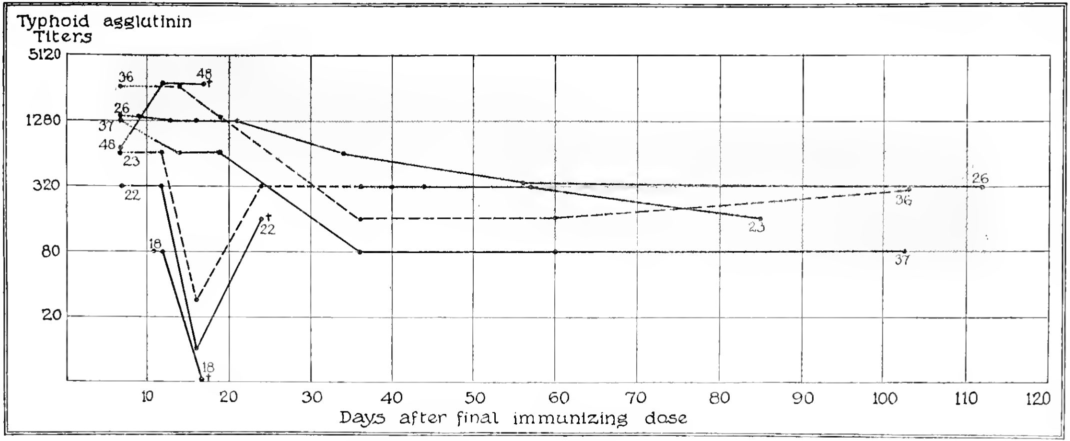
Animal No.	Condition.	Serum of.	Interval between tests.	First titer.	Second titer.
			days		
22	Double adrenalectomy almost complete.	Dec. 27, 1916	35	1:30	1:640
		Jan. 4, 1917	26	1:320	1:1,280
23	Right adrenal removed.	Dec. 27, 1916	35	1:160	1:640
		Jan. 4, 1917	26	1:320	1:320
	Three-fourths of left adrenal removed.	Jan. 16, 1917	15	1:320	1:640
		" 20, 1917	7	1:320	1:320
		Mar. 5, 1917	7	1:160	1:320
24	Normal.	Dec. 27, 1916	35	1:1,280	1:2,560
		Jan. 4, 1917	26	1:1,280	1:2,560
	Control operation.	Jan. 16, 1917	15	1:1,280	1:1,280
		" 20, 1917	7	1:640	1:1,280
		Mar. 5, 1917	7	1:160	1:320
2	Double adrenalectomy.	Mar. 5, 1917	7	1:160	1:640
		" 12, 1917	14	1:640	1:640
39	Normal.	Mar. 5, 1917	7	1:160	1:160

guinea pig immunized, and the second gland or part of it taken at a later date. These two sets of experiments, in which the effect of partial adrenalectomy was studied in animals previously or subsequently immunized to *Bacillus typhosus*, form a group in which all the control animals may serve as a basis for comparison with those on which the extirpation of adrenal tissue was performed. The agglutinin curves of the control animals showed considerable variations

due to individual reaction, so that a plot of all the normal curves gives a confusion of lines within rather wide limits on the chart. Obviously comparison of the antibody curve of an adrenalectomized animal with its own control alone might indicate differences in reaction not due essentially to the loss of adrenal tissue. Therefore it seems best simply to outline an area which covers all the variations in reaction found in normal guinea pigs. Against this normal area the separate curves of agglutinin formation in the experimental animals may be charted. Following this method Text-fig. 1 shows the curves of seven guinea pigs which were adrenalectomized subsequent to immunization. It will be seen that the titers of all but three of them fall practically within the limits of normal variation. The findings in the exceptional cases require special comment.

Immediately after operation the agglutinin titers of the sera of these animals appeared to drop sharply almost to zero, with as sharp a rise later in the two guinea pigs which survived. No similar drop was apparent in the titer of the control. These readings were made on the fresh inactivated sera the same day they were taken. When observations on other animals failed to confirm this finding, these sera, which had been kept in the dark at ice box temperature for about a month, were reexamined, and now gave titers more nearly corresponding to those of the later experiments. The differences in titer are shown in Table I, with the record of subsequent analyses in which sera were studied while fresh, and again after standing several days. In a number of instances it will be seen that they agglutinated the Sen strain in higher dilutions on reexamination. Since this observation was made on normal as well as adrenalectomized guinea pigs the difference must be sought in some other factor, which would account for the apparent inhibition in fresh sera. Moreover, this initial inhibition does not appear with regularity. For uniformity it is necessary to use the agglutination titers from the fresh sera in drawing conclusions from the experiments, but it is apparent that disturbing factors are latent in the results.

With the exceptions noted above, these experiments indicate that adrenalectomy subsequent to immunization has no significant effect upon the curve of typhoid agglutinin formation in guinea pigs. Similar results were found in the experiments charted in Text-fig. 2, in which, against the normal background are seen the agglutination curves of seven guinea pigs which were immunized subsequent to adrenalectomy. Their reactions to immunization fall substantially within normal limits.



.... Period before operation.

— — — — One adrenal removed.

———————— One, and most of the other adrenal removed.

The shaded area covers the variations in titers of the controls.

TEXT-FIG. 1. Typhoid agglutinin titers of animals immunized before adrenalectomy.

Partial adrenalectomy, with removal of a single gland, or of one gland and as much of the other as can be taken with impunity, appears to have no influence upon the formation of typhoid agglutinins in guinea pigs.

Series II.

Cole[7] has described a late effect of immunization which persists after demonstrable immune bodies have disappeared from the blood.

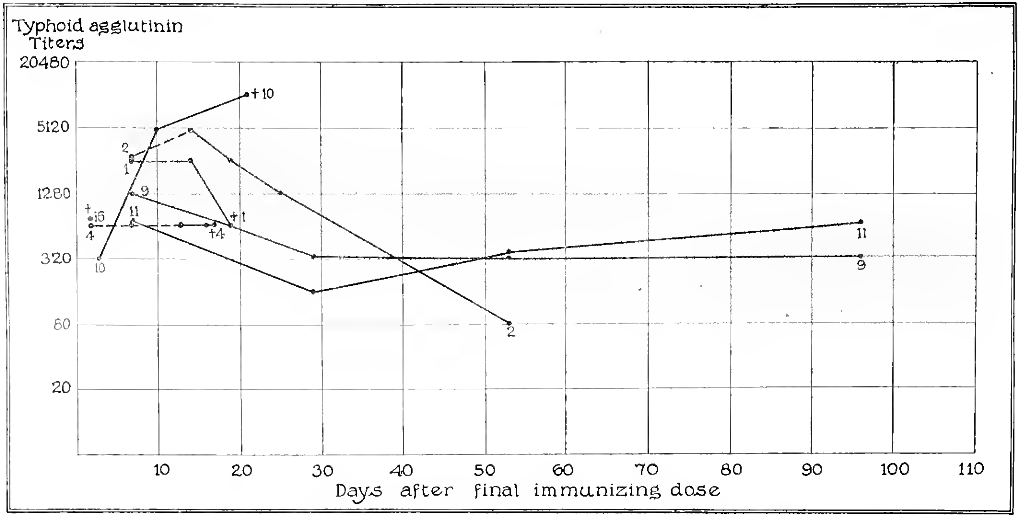
TABLE II.

Rise in Agglutinin Titers of Adrenalectomized and Control Guinea Pigs on Reinjection of Typhoid Vaccine.

Animal No.	Previous history.	Serum agglutinin titer on Mar. 5, 1917.		Serum agglutinin titer on Mar. 12, 1917.	Serum agglutinin titer on Apr. 24, 1917.
23	Adrenalectomized; immunized 84 days before.	1:160		1:320	1:80
24	Operated control; immunized 84 days before.	1:160	Mar. 5, 1917. Injected intraperitoneally 0.05 mg. of typhoid vaccine.	1:640	1:160
2	Immunized 53 days before; then adrenalectomized.	1:80		1:640	1:320
39	Normal; immunized 53 days before.	1:160		1:320	1:160
60	Normal.	0		1:20	1:20
61	"	0		0	1:10

Reinjection of previously immunized animals with a minute dose of the original antigen, an amount which has no effect upon normal animals, causes a sharp rise in the antibody curve. This effect is interpreted as due to a latent tissue sensitization. To test the presence of this phenomenon in adrenalectomized animals, two of the survivors from the experiments already described, with their controls, and with two normal guinea pigs, were injected intraperitoneally with 0.05 mg. of the typhoid vaccine used for the original immunization. At this time the animals from the earlier experiments

[7] Cole, R. I., Experimenteller Beitrag zur Typhusimmunität, *Z. Hyg. u. Infectionskrankh.*, 1904, xlvi, 371.

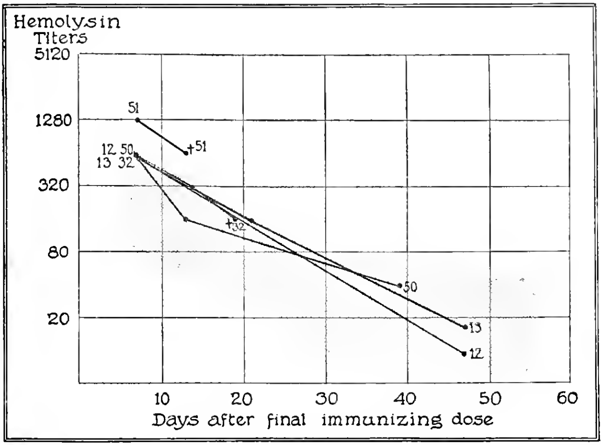


TEXT-FIG. 2. Typhoid agglutinin titers of animals immunized after adrenalectomy.

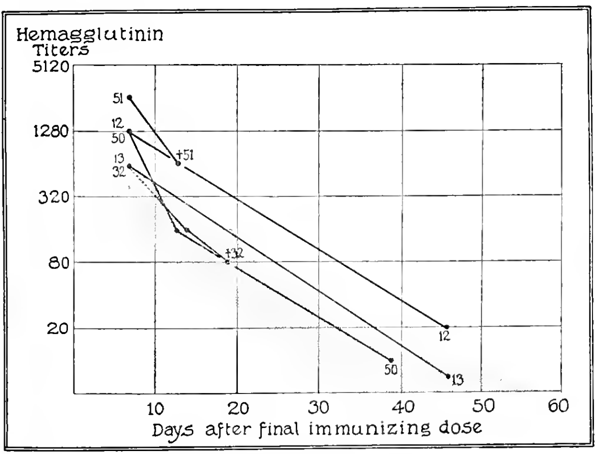
showed typhoid agglutinins in dilutions of 1:80 to 1:160 of their sera. The normal controls showed no antibodies. 7 days later the titers of the previously immunized guinea pigs had risen to 1:320 to 1:640, while the single dose of 0.05 mg. of vaccine had resulted in agglutinin formation to the extent of 1:20 in one control. These findings, and the results of a subsequent titration are found in Table II. The tissue sensitization occurred normally in the partially adrenalectomized guinea pigs and their previously immunized controls.

Series III.

With the technique already described a smaller number of experiments was made on guinea pigs immunized against hen corpuscles. Hemagglutinins and hemolysins were studied in animals adrenalectomized before or after immunization in experiments similar to those in Series I. Although the initial antibody titers corresponded roughly to those obtained with typhoid vaccine, the hemagglutinins and hemolysins disappeared from the circulating blood much more rapidly, so that the antibody content was reduced almost to nothing in the course of 2 months. The results of titrations at intervals are shown in Text-figs. 3 and 4, in which, against a background of normal variations are plotted the curves of the adrenalectomized animals. Two of these, namely Guinea Pigs 12 and 13, were adrenalectomized before immunization, losing one-half the right gland 43 days, and the left gland 7 days before the first injection of corpuscles. The other adrenalectomized animals, namely Guinea Pigs 32, 50, and 51, were first immunized and then operated upon 7 to 10 days after the final injection. The high initial agglutinin titers of Guinea Pigs 50 and 51 are due to four, instead of the usual three injections. Of the three animals in this experiment, those with the highest titers were chosen for operation. The control animal, Guinea Pig 52, had an initial hemagglutinin titer of 1:640. The slopes of the curves for Guinea Pigs 50 and 51 are seen to parallel the normal curve, although the titers for Guinea Pigs 50 and 51 do not fall within the normal limits. The charted results show that adrenalectomy has no essential influence upon the production or gradual diminution of hemolysins and hemagglutinins in guinea pigs.



TEXT-FIG. 3. Hemolysin titers of adrenalectomized animals. Guinea Pigs 12 and 13 were adrenalectomized before immunization and Guinea Pigs 32, 50, and 51 after immunization.



TEXT-FIG. 4. Hemagglutinin titers of adrenalectomized animals. Guinea Pigs 12 and 13 were adrenalectomized before immunization and Guinea Pigs 32, 50, and 51 after immunization.

Series IV.

As with the survivors from the experiments with typhoid agglutinins, the guinea pigs immunized against hen corpuscles were given a second small injection of the foreign blood cells after antibodies had almost disappeared from their sera. With two normal controls, and

TABLE III.

Rise in Hemolysin and Hemagglutinin Titers of Previously Immunized Guinea Pigs on Reinjection with Hen Corpuscles.

Animal No.	Previous operative history.	Previous immunization.	Hemolysins.			Hemagglutinins.		
			Time before injection.	Time after injection.		Time before injection.	Time after injection.	
			5 days.	7 days.	45 days.	5 days.	7 days.	45 days.
13	Double adrenalectomy 68 days before.	52 days before.	1:10	1:160	1:40	0	1:20	1:20
31	Double adrenalectomy 4 days before.	61 " "	0	1:320	1:20	0	1:40	1:10
43	Double adrenalectomy 4 days before.	52 " "	1:10	1:320	1:20	0	1:80	1:20
50	Double adrenalectomy 37 days before.	44 " "	1:40	1:320	1:40	1:10	1:40	1:40
55	Double adrenalectomy 3 days before.	40 " "	1:40	1:160	1:40	0	1:40	1:20
54	Right adrenal removed 3 days before.	40 " "	1:10	1:320		0	1:40	
33	Control operation 51 days before.	61 " "	1:40	1:160	1:40	0	1:40	1:20
44	None.	52 " "	1:10	1:160	1:40	0	1:40	0
52	"	44 " "	1:40	1:320	1:80	1:10	1:40	1:10
53	"	40 " "	1:20	1:20	1:10	0	1:20	1:10
62	None; normal.	None.	0	0	0	1:80	1:40	1:10
63	" "	"	0	0	0	0	0	0

four control guinea pigs previously immunized, six partially adrenalectomized animals were injected intraperitoneally with 0.05 cc. of washed hen corpuscles. One of the adrenalectomized guinea pigs had been operated upon before the previous immunization. The other four had lost adrenal tissue at varying intervals after immunization. Although the minute dose of antigen given did not stimu-

late antibody production to the original level, the adrenalectomized and all but one of the control animals which had been previously immunized responded with hemagglutinin titers of 1: 20 to 1: 80, and hemolysin titers of 1: 160 to 1: 320 on the 7th day, whereas the normal control animals, for which this was the first injection, failed to show demonstrable increase of antibodies. Control Guinea Pig 62 with an initial hemagglutinin titer of 1: 80 before injection gave subsequent titers of 1: 40 and 1: 10, showing no effect from the injection. 5 weeks later the hemolysins of most of the animals had fallen below 1: 80, and the hemagglutinins below 1: 40. These results are given in Table III.

In addition to the experiments described above on guinea pigs, three rabbits were partially adrenalectomized and tested for *in vivo* agglutinins, as described by Bull.[8] Typhoid bacilli injected on the 4th or the 24th day after adrenalectomy were clumped and removed from the blood stream with the same rapidity and completeness as in normal animals. The blood of these rabbits was then tested for natural typhoid agglutinins by the usual method *in vitro*. The titers ranged from 1: 16 to 1: 128, showing that neither adrenalectomy nor the *in vivo* agglutination had removed these antibodies from the blood.

SUMMARY.

By careful aseptic operation it was found possible to remove approximately three-quarters to seven-eighths of the adrenal tissue of guinea pigs without causing symptoms of adrenal insufficiency. Guinea pigs were immunized to *Bacillus typhosus* or to hen corpuscles at varying intervals before or after the operation, and the curves of antibody formation traced for 2 to 3 months after immunization. Comparisons with the antibody curves of control animals similarly immunized fail to show that the adrenalectomy had any influence upon the rise or persistence of antibodies in the blood.

For the purposes of the study it was not deemed necessary to produce an acute adrenal insufficiency. If the adrenal glands were the site of antibody formation or played an essential part in immunity processes, it does not seem probable that the small remainder of

[8] Bull, C. G., The agglutination of bacteria *in vivo*, *J. Exp. Med.*, 1915, xxii, 484.

adrenal tissue left *in situ* to sustain life would affect quantitatively the antibody response to a given antigen injection as do the entire normal glands. We therefore interpret the experiments to indicate that not only are the adrenal glands not one of the important sources of typhoid agglutinins, or of hemagglutinins or hemolysins, but they play no essential part in the mechanism by which these antibodies are produced and maintained in the body.

ÆSTIVO-AUTUMNAL MALARIA. THE EXTRACELLULAR RELATION OF THE CRESCENTIC BODIES TO THE RED CORPUSCLE AND THEIR METHOD OF SECURING ATTACHMENT.*

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PLATES 33 TO 35.

(Received for publication, December 31, 1917.)

Æstivo-Autumnal Parasite.

Æstivo-autumnal parasites are extracellular throughout their life cycle; they migrate from one red corpuscle to another, destroying several in the course of their development. They pass through a sexual cycle in the human host,[1] with the formation of flagella by the microgametocyte, fertilization of the macrogamete, and its subsequent segmentation. I have seen these phases many times. The great difficulty in working out the phases in the æstivo-autumnal infections is that since the infection is usually so serious, one hardly feels justified in withholding treatment of the infected individual for the period of time necessary for the parasites to become very numerous, or, if already numerous, for the time required for a protracted study of them during their developmental phases. (The developmental and sexual phases rarely appear in the peripheral blood unless the patient has a very heavy infection.) It requires many hours for the complete examination of even one film of the patient's blood.

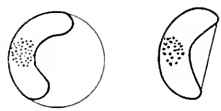
Crescentic Bodies.

The life phase of the æstivo-autumnal parasite is represented by the characteristic crescentic bodies. They develop in gradual stages

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] Rowley-Lawson, M., The æstivo-autumnal parasite: its sexual cycle in the circulating blood of man, with a description of the morphological and biological characteristics of the parasite, *J. Exp. Med.*, 1911, xiii, 263.

from the small ring-form parasites as round bodies, finally opening out into the crescent form. Several days are required for their development, which usually takes place in the internal organs of the host. The crescent may assume other forms, such as fusiform, ovoid, and round. Many of the so called round and ovoid bodies are (*a*) crescents viewed from their convex side; (*b*) crescents bent on themselves; (*c*) adult crescents contracted into round bodies and ovoids. The contracted round bodies and ovoids are usually seen at the edges of films made on slides and cover-slips. By the examination of many specimens one soon learns to distinguish between the developing (round body) crescent and the round form assumed by the adult crescent.



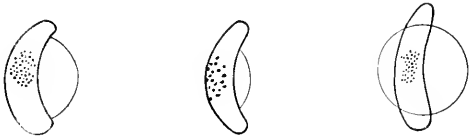
a b

TEXT-FIG. 1, *a* and *b*. The appearances one would get if the parasite were within the corpuscle; that is, the outline of the decolorized corpuscle would correspond to the convexity of the crescentic body. One would not expect it to embrace the ends of the crescent, and then bulge out from the concavity of the crescent in the curved line, as shown in Text-fig. 2, *a*, *b*, and *c*. It would be more likely to present an appearance such as is shown in Text-fig. 1, *b*.

Extracellular Relation of the Crescent to the Red Corpuscle.—The crescentic bodies are generally believed to be within the substance of the red corpuscle in spite of the many evidences, easily demonstrated, that they are attached to its external surface. Even if one could not understand the method by which this attachment was secured, the relation of the "bib" to the parasite should enable one to see that they are not within the corpuscle. For instance, if they were within the corpuscle, one would expect to get such appearances as are shown in Text-fig. 1. As a matter of fact, the appearances most commonly met with and illustrated are those given in Text-fig. 2.

Attachment of the Crescent to the Red Corpuscle.—The crescent follows in general the same method of attachment to the corpuscle as

do the parasites of the other malarial infections.[2] They encircle with their cytoplasm mounds of hemoglobin substance, which assists them to maintain their rather precarious position on the surface of the corpuscle while they dissolve and digest the hemoglobin. The mounds of hemoglobin to which the corpuscles are attached may be seen protruding through the cytoplasm of the body proper of the crescent as well as at the periphery (Figs. 1 to 105). One might say that the parasite attaches itself to the corpuscle in two ways: (*a*) encircling with its body protoplasm surface mounds of hemoglobin; (*b*) encircling with pseudopodia arising from the cytoplasm peripheral mounds of hemoglobin.



a b c

TEXT-FIG. 2, *a*, *b*, and *c*. The crescent wraps itself around the red corpuscle, and proceeds to decolorize it. The so called "bib" is the decolorized corpuscle. When the bib is present it practically always comes from the concavity of the crescentic body as shown in *a*, *b*, and *c*. Ordinarily the bib appears only on one side of the crescent, but occasionally it may be seen on both sides as is schematized in *c*. In these instances the outline of the decolorized corpuscle can usually be traced through the substance of the crescent.

Surface Mounds.—The mounds of hemoglobin substance protruding through the protoplasm of the parasite do not seem to alter the general outline of the crescentic body. In many instances where the apex of the surface mound extends beyond the periphery of the crescentic body, the outline of the crescent may be traced beneath the transparent mound. The mounds protrude through various parts of the body of the crescent, some of them protruding where the nucleoplasm is supposed to be; in fact, in rare cases the chro-

[2] In previous publications I have explained and illustrated the method by which the young parasites of æstivo-autumnal infections (Figs. 1 to 7), as well as the parasites of tertian infections (Figs. 8 to 10), secure their attachment to the external surface of red corpuscles.

matin granules may be seen outlining the hemoglobin mound at its base (Figs. 21 and 79 at oo). Frequently the pigment granules may be seen outlining the base of a hemoglobin mound (Figs. 11, 17, and 44 at x).

Peripheral Mounds.—These are seen along the edges of the crescentic body, and do not appear to have protruded through the body proper of the parasite. I believe that these peripheral mounds are encircled by pseudopodia.

Attaching Pseudopodia.—I do not know whether these pseudopodia are used for the purpose of capturing their prey as well as for the purpose of securing it after it has been captured. They arise from the cytoplasm of the parasite, staining similarly, and may show definitely, especially in specimens where the hemoglobin mounds are seen. As one would expect, considering their purpose, they may be seen either in the form of loops (Figs. 43, 58, 60, 70, 98, and 105 at o), or as strings of cytoplasm (Figs. 21, 34, 56, 57, 59, 79, 97, and 98 at o). The large amount of cytoplasm which may enter into the formation of these pseudopodia is surprising (Figs. 59, 60, 70, 98, 99, and 105).

I have observed these mounds, especially the peripheral mounds, in fresh preparations. This has led me to believe that perhaps some of the small round bodies seen by observers about the periphery of the crescent might be hemoglobin mounds.

Celli and Guarnieri[3] note that "these crescent and ovoid forms may show small round bodies—buds, as it were—about the periphery, one or more in number." Thayer[4] states: "We may observe in certain instances the protrusion of small delicate, bud-like bodies which are cut off from the cell." Celli and Guarnieri suggest that these bodies may represent a method of reproduction, while Thayer states that it is probably a degenerative process. Sforza,[5] judging from certain observations on the staining reactions of crescents, concludes that "the greater part of the crescentic body is nothing more or less than the degenerate red corpuscle." To reach this conclusion, could he have seen the hemoglobin mounds protruding through the crescentic body?

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[3] Celli and Guarnieri, quoted from Thayer, W. S., and Hewetson, J., The malarial fevers of Baltimore, *Johns Hopkins Hosp. Rep.*, 1895, v, 162.

[4] Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 73.

[5] Sforza, quoted from Thayer and Hewetson, The malarial fevers of Baltimore, *Johns Hopkins Hosp. Rep.*, 1895, v, 169.

Decolorization of Corpuscular Mounds by Parasitic Action. Vacuolization.

As a rule, mounds of hemoglobin substance are rather rapidly decolorized (dissolved and assimilated) by the parasite. This may be demonstrated by the examination of a large series of films taken in immediate succession. After decolorization of the surface mounds has taken place, the appearance of the parasite corresponds to what has often been described and pictured as "vacuolization." Mounds in the process of decolorization by the action of the parasite are frequently seen (Figs. 62 to 69). I have observed vacuoles in the living parasite many times and have never considered the process a degenerative one. The vacuoles vary in size and shape and two or more may run together to make one.

The idea that vacuolization is synonymous with degeneration seems to be well fixed in the minds of many observers. Canalis[6] pictures a crescent with vacuoles, describing it as a crescent showing degeneration. Antolisei and Angelini[7] describe "degenerate vacuolating forms which represent the death of the parasite." Mannaberg[8] writes: "In fresh preparations appearances are sometimes seen in the crescents which must be considered to be processes of degeneration. They consist of the appearance of clear circles and spots, which alter their shape under the observer's eye." Marchiafava and Bignami[9] write: "We may also see the process of vacuolization of the crescent bodies as well as of the ovoid and round ones," describing it as a degenerative alteration. Celli and Guarnieri[3] note "vacuolic degeneration of the crescentic forms." Manson[10] writes that the protoplasm of the crescent "shows vacuolation and other signs of degeneration."

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[6] Canalis, P., Studi sulla Infexione malarica Sulla varièta parassitaria delle forme semilunari di Laveran e sulle febbri malariche che da esse dipendono, *Arch. sc. med.*, 1890, xiv, 75, and Plate C, Fig. 11.

[7] Antolisei and Angelini, quoted from Thayer and Hewetson, The malarial fevers of Baltimore, *Johns Hopkins Hosp. Rep.*, 1895, v, 164.

[8] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and other observers; Translation by Felkin, R. W., London, 1894, 287.

[9] Marchiafava, E., and Bignami, A., Malaria, in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 47.

[10] Manson, P., Tropical diseases: a manual of the diseases of warm climates, London, Paris, New York, and Melbourne, 2nd edition, 1900, 14.

Thayer[11] states: "Vacuolization of the crescentic, ovoid, and round bodies is not very uncommon. This is usually associated with a diminution of the refractiveness of the parasite and often with a loss of regular outline. The vacuoles are small, but may vary considerably in size, sometimes becoming confluent and larger. The process is evidently degenerative." Thayer and Hewetson[12] state that the "vacuolic degeneration" of crescentic forms is a process which has previously been described by Laveran.

Vacuoles.

Contractile or Pulsatile Vacuoles.—In protozoa the vacuole is not a vacant space. In the fresh water amebæ, in addition to food vacuoles, one may see contractile or pulsatile vacuoles. These vacuoles are exceptional in size and constancy of position. They are usually excretory organs, containing a combination of fluid and gas. At fairly regular intervals they may be seen to contract until they disappear, reforming slowly. But it is not the contractile or pulsatile vacuoles with which we have to do in connection with the malarial parasite, but with the food vacuoles.

Food or Nutritive Vacuoles.—These are usually regarded as of temporary character. They contain liquid, not gas, probably a chemical ferment which dissolves the hemoglobin and makes it available for utilization by the parasite. The parasite assimilates what it needs for nutrition, the waste products being converted into pigment, which is excreted when the parasite segments.

The fresh water ameba resembles the malarial ameba in some respects. It secures its prey by means of pseudopodia which surround the prey, the pseudopodium of the ameba uniting to enclose it within the boundary of its protoplasm, in this way forming a so called nutritive vacuole with the prey as the inclusion. The ameba then proceeds to dissolve the inclusion, absorbing the dissolved material into its substance, storing the reserves, and throwing off the waste products.

I have seen much the same process occur with the mononuclear leukocyte of human blood.[13] The leukocyte put out pseudopodia from

[11] Thayer, Lectures on the malarial fevers, New York, 1897, 72.

[12] Thayer and Hewetson, The malarial fevers of Baltimore, *Johns Hopkins Hosp. Rep.*, 1895, v, 162.

[13] Rowley, M. W., A fatal anæmia with enormous numbers of circulating phagocytes, *J. Exp. Med.*, 1908, x, 78.

its cytoplasm, which captured and included a polynuclear leukocyte, the pseudopodia uniting to form the wall of the nutritive vacuole with the polynuclear leukocyte within it. The mononuclear cell then proceeded to dissolve the inclusion and the gradual disappearance of the structure of the polynuclear leukocyte could be watched.

The vacuoles seen in connection with the malarial parasite are what one would expect to find and are not an indication of degeneration. Grassi[14] shows them in crescentic bodies, Schaudinn[15] pictures vacuoles in the "*ookinete*," and Ruge[16] illustrates them in a proteosoma. Even the decolorized mound ("achromatic area") seen in connection with the young parasite must be a converted nutritive vacuole since the parasite has dissolved and assimilated the hemoglobin which was enclosed within its pseudopodium.

Is it possible that the malarial parasite, like the fresh water ameba, may secrete reserves which would enable it to withstand lack of food for a short time? If this were so, it would suggest what we already know to be necessary—vigorous and long continued treatment.

SUMMARY.

Æstivo-autumnal parasites, including the crescentic bodies, are always extracellular; that is, they are attached to the external surface of the red corpuscles.

Crescentic bodies attach themselves to the red corpuscles just as the younger parasites do, by encircling, with their cytoplasm, mounds of hemoglobin substance. These hemoglobin mounds may be seen protruding through various portions of the crescentic bodies, as well as at the periphery of the parasites. The base of the mounds is occasionally outlined by the chromatin or pigment granules.

The hemoglobin mounds protruding through the body proper of

[14] Grassi, B., Die Malaria, Studien eines Zoologen, Jena, 2nd edition, 1901, Figs. 11, 12, 14 to 17, 20, 21, 24, 25, and 28.

[15] Schaudinn, F., Studien über krankheitserregende Protozoen. II. *Plasmodium vivax* (Grassi & Feletti), der Erreger des Tertianfiebers beim Menschen, *Arb. k. Gsndhtsamte.*, 1903, xix, 169, and Plate 4, Figs. 37, 38, 39, and 40.

[16] Ruge, R., Einführung in das Studium der Malariakrankheiten mit besonderer Berücksichtigung der Technik. Ein Leitfaden für Schiffs- und Colonialärzte, Jena, 1901, Plate 1, Fig. 41.

the crescentic bodies do not seem to alter the general outline of the parasites. The outline of the parasites may be traced through the transparent mounds.

Whenever attaching pseudopodia are observed they are seen to arise from the cytoplasm of the parasites and may be in the form of loops or strings.

When the crescents are attached they proceed to dissolve the hemoglobin to make it available for utilization, assimilating what is required for nutrition, the waste product being in the form of pigment granules.

After the hemoglobin mounds, to which the crescents are attached, have been decolorized by parasitic action, an appearance is obtained which has been described by most observers as vacuolization of the crescentic body. These observers believe the picture to be one of degeneration.

The decolorized mounds or vacuoles ("achromatic areas") seen in connection with malarial parasites correspond to the nutrition vacuoles of the common amebæ, and possibly the malarial parasite may, like these amebæ, secrete reserve food.

EXPLANATION OF PLATES

Plate 33.

Magnification, × 1,690.

Figs. 1 to 7. Young æstivo-autumnal parasites attached to peripheral mounds of hemoglobin substance. The pseudopodia of the parasites have encircled the mounds at their base.

Figs. 8 to 10. Adult tertian parasites attached to peripheral corpuscular mounds.

Figs. 11 to 35. Crescentic bodies of æstivo-autumnal infections attached to peripheral and surface mounds of hemoglobin substance. In Figs. 11, 17, 26, and 28 the pigment granules are seen outlining the hemoglobin mounds at their base, at x. In Fig. 21 the chromatin granules may be seen outlining the base of the mound at oo. In Figs. 21 and 34 the pseudopodium may be seen at o.

Figs. 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 33, and 35 correspond to Figs. 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and 90.

Examination of these pictures with a magnifying glass will show definitely the mounds of hemoglobin substance protruding through the bodies of the crescents.

PLATE 34.

Magnification, × 1,690.

FIGS. 36 to 55. Crescentic bodies attached to peripheral and surface mounds of hemoglobin substance. Fig. 43 shows at o the pseudopodium in the form of a loop. Fig. 44 shows at x pigment granules surrounding the base of the hemoglobin mound. Fig. 45 shows at xx mounds of hemoglobin substance, which was stained a deep pink, reproducing black in the photograph.

Figs. 37, 41, 44, 46, 49, and 55 correspond to Figs. 91, 92, 93, 94, 95, and 96.

FIG. 56. A crescent body attaching to a red corpuscle by means of its pseudopodia, seen at o. This figure corresponds to Fig. 97.

FIG. 57. A crescent attached to a partially decolorized corpuscle. The mounds of hemoglobin substance may be seen and a pseudopodium is seen at o.

FIG. 58. A crescent with an attaching loop of cytoplasm showing at o.

FIG. 59. A crescentic body showing corpuscular mounds and an attaching pseudopodium at o. This figure corresponds to Fig. 98.

FIG. 60. The attaching pseudopodia may be seen at o in the form of loops. This figure shows a large amount of cytoplasm entering into the pseudopodia. This figure corresponds to Fig. 99.

FIG. 61. The corpuscle to which the parasite is attached shows well here, and a careful examination of the parasite will show the surface mounds. This figure corresponds to Fig. 100.

FIG. 62. Here the hemoglobin mounds are showing decolorization. The entire upper end of the parasite is occupied by a partially decolorized mound of hemoglobin protruding through the cytoplasm of the parasite.

FIG. 63. Here one sees the beginning of what is popularly termed vacuolization. The parasite is dissolving and digesting the hemoglobin and the digestive vacuoles can be seen more clearly than when they are filled with the well stained hemoglobin substance. This figure corresponds to Fig. 101.

FIGS. 64 and 65. The dissolving of the hemoglobin in the vacuole, the operation being a little more advanced in Fig. 65. Fig. 64 corresponds to Fig. 102.

FIG. 66. The strands of cytoplasm of the parasite may easily be seen between the vacuoles. The vacuoles are oval in shape and the included hemoglobin almost decolorized. This figure corresponds to Fig. 103.

FIGS. 67 and 68. Definite vacuoles in the body of the parasites. Fig. 68 corresponds to Fig. 104.

FIG. 69. A large vacuole in the parasite.

FIG. 70. The crescentic body here shows that it is attached to corpuscular mounds and a loop arrangement of cytoplasm is seen extending from one end of the parasite to the other end, at o. This figure corresponds to Fig. 105.

PLATE 35.

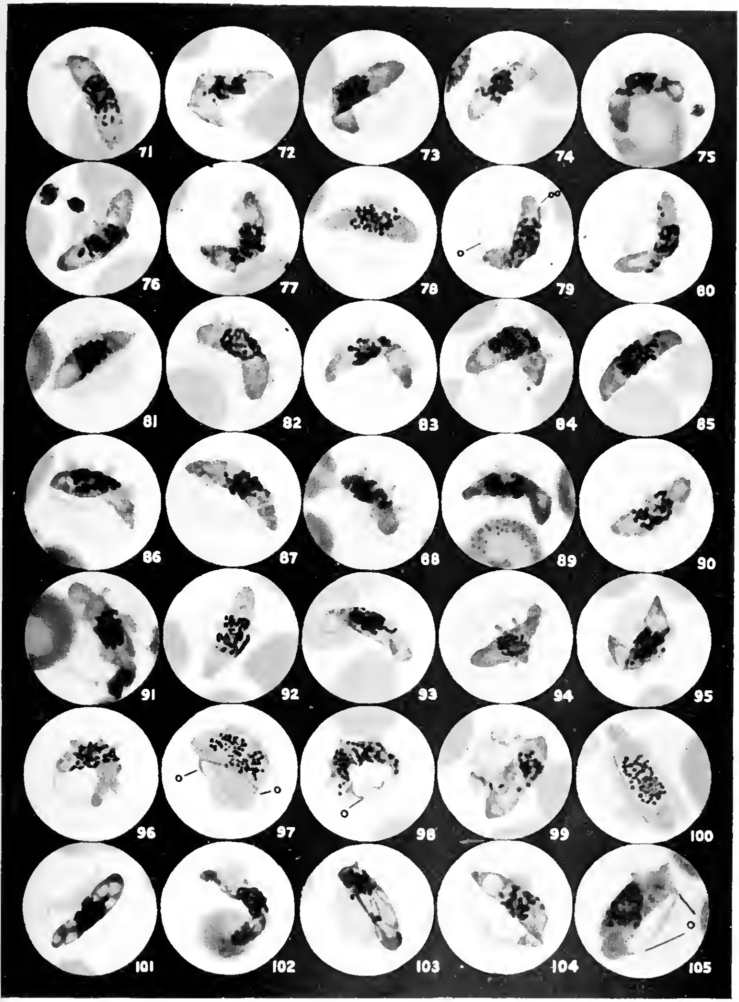
Magnification, × 1,690.

FIGS. 71 to 105. These pictures are colored photographs of certain parasites shown in the black and white reproductions. They show the parasites attached to peripheral and surface mounds of hemoglobin substance. The attaching pseudopodia and the nutritive vacuoles of the parasites can also be seen.

Fig. 71 corresponds to Fig. 12.
Fig. 72 corresponds to Fig. 13.
Fig. 73 corresponds to Fig. 14.
Fig. 74 corresponds to Fig. 15.
Fig 75 corresponds to Fig. 16.
Fig. 76 corresponds to Fig. 18.
Fig. 77 corresponds to Fig. 19.
Fig. 78 corresponds to Fig. 20.
Fig. 79 corresponds to Fig. 21.
Fig. 80 corresponds to Fig. 22.
Fig. 81 corresponds to Fig. 24.
Fig. 82 corresponds to Fig. 25.
Fig. 83 corresponds to Fig. 26.
Fig. 84 corresponds to Fig. 27.
Fig. 85 corresponds to Fig. 28.
Fig. 86 corresponds to Fig. 29.
Fig. 87 corresponds to Fig. 30.
Fig. 88 corresponds to Fig. 31.
Fig. 89 corresponds to Fig. 33.
Fig. 90 corresponds to Fig. 35.
Fig. 91 corresponds to Fig. 37.
Fig. 92 corresponds to Fig. 41.
Fig. 93 corresponds to Fig. 44.
Fig. 94 corresponds to Fig. 46.
Fig. 95 corresponds to Fig. 49.
Fig. 96 corresponds to Fig. 55.
Fig. 97 corresponds to Fig. 56.
Fig. 98 corresponds to Fig. 59.
Fig. 99 corresponds to Fig. 60.
Fig. 100 corresponds to Fig. 61.
Fig. 101 corresponds to Fig. 63.
Fig. 102 corresponds to Fig. 64.
Fig. 103 corresponds to Fig. 66.
Fig. 104 corresponds to Fig. 68.
Fig. 105 corresponds to Fig. 70.

(Lawson: Crescentic bodies in æstivo-autumnal malaria.)

(Lawson. Crescentic bodies in æstivo-autumnal malaria.)



(Lawson: Crescentic bodies in æstivo-autumnal malaria.)

ÆSTIVO-AUTUMNAL PARASITES. MULTIPLE INFECTION OF RED CORPUSCLES AND THE VARIOUS HYPOTHESES CONCERNING IT.*

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PLATES 36 TO 39.

(Received for publication, February 1, 1918.)

Malarial Parasites.

There are three species of malarial parasites, each with its special morphological and biological characteristics. They are: (1) tertian parasites, (2) quartan parasites, and (3) æstivo-autumnal parasites. It is with the last variety that this paper deals.

Æstivo-Autumnal Parasite.—The young forms of these parasites are very much like those of the tertian and quartan infections, except that, as a rule, the parasites of the æstivo-autumnal infections are relatively smaller than those of the tertian and quartan infections. They are more delicate in appearance, have a more clean-cut outline and a smaller chromatin mass. One should learn to distinguish these morphological differences, as the young parasite may be the only form present at the time of the examination of the blood. The æstivo-autumnal parasites produce the gravest manifestations of the malarial infections so that an early diagnosis may be of the greatest importance.

Multiple Infection of Red Corpuscles with Young Parasites.

Multiple infection of red corpuscles with young parasites is seen in all malarial infections and it is not a rare occurrence in the æstivo-autumnal infections. In films from the circulating blood I have seen from two to seven young parasites on a corpuscle (Figs. 1 to 75).

* Aided by a grant from The Rockefeller Institute for Medical Research.

The occurrence is always accidental and has no significance other than that, if the instances are numerous, it usually means a heavy infection. As a rule, the number of parasites attached to individual corpuscles increases in direct proportion to the severity of the infection; therefore one would not expect to find three, four, or five parasites on a corpuscle in a film where the parasites present were few in number. This fact in itself should suggest that instances of multiple infection have no significance; but various theories have been formulated to explain certain examples.

Theories as to Multiple Infection.—The position of the attached parasites in relation to each other has, apparently, suggested certain theories, such as conjugation ("syngamy," "cytogamy"). The fusion of two cells, cytoplasm to cytoplasm, and chromatin to chromatin, to form a new individual, is a process which has been described frequently in connection with certain protozoa.

Mannaberg[1] was one of the first observers to formulate a theory as to the conjugation of the young amebæ of æstivo-autumnal infections. Two or more parasites were seen attached to adjacent hemoglobin mounds. They were attached so closely together that a portion of the cytoplasm of one parasite was overlying a portion of the cytoplasm of the adjacent parasite. This appearance (Figs. 11 to 30 and 119 to 125) was interpreted by Mannaberg as conjugation. He believed that a fusion of the cytoplasm had taken place, resulting in transitional forms in the formation of the crescents. It would seem that it did not make any difference whether two, three, or four of these young parasites had united to form a crescent, for he states: "I have observed two, or more rarely three, of these parasites may lie closely adhering to one another," and he speaks of "these conglomerate parasites, consisting of two to four specimens." Wright[2] states: "My observations appear to support those of Mannaberg in regard to the genesis of the crescent—the syzygium—from a corpuscle doubly infected by parasites," and "In the stained specimens the syzygies appear to be in the act of conjugation."

As a matter of fact these parasites never unite. The same appearance may be seen in tertian infections.

[1] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and other observers; Translation by Felkin, R. W., London, 1894, 289.

[2] Wright, H., The malarial fevers of British Malaya, *Studies from Institute for Medical Research, Federated Malay States*, Singapore, 1901, i, 4.

Multiple Infection of Corpuscular Mounds.

I have seen from two to five young parasites attached to one corpuscular mound (Figs. 1 to 9, 19, 20 24, 25, 27, 29 to 35, and 37 to 70). Multiple infections of mounds have no significance. The heavier the infection, the more frequently it is seen. The parasites attached to one mound may be in similar or in varying stages of development. The cytoplasm of each parasite is in contact with the hemoglobin mound, and each parasite has a share in its destruction.

Occasionally one finds two parasites attached to one mound so as to give the appearance of a developing crescent (Figs. 31 to 35, 126, and 127), and the same appearance may be seen in tertian infections (Fig. 35). In comparing these instances of æstivo-autumnal and tertian infections of mounds, note the relatively larger size of the chromatin masses of the tertian parasites.

Many theories have been formulated as to the significance of the appearance where two or more parasites encircled one mound of hemoglobin substance, especially by observers who believed the parasites to be within the substance of the corpuscle.

The youngest form of the æstivo-autumnal parasite to attach itself to the red corpuscle may require its entire cytoplasm to encircle a hemoglobin mound. When one of these parasites is so attached, it appears as a ring-form of a delicate, thread-like structure, more or less uniform in size throughout its circumference. If two or more of these tiny parasites encircle the one hemoglobin mound, the cytoplasm of one parasite superimposed over the cytoplasm of the other parasite or parasites, and the chromatin masses separated or lying close together, as accident may direct, the appearance of a single ring with more than one mass of chromatin is obtained (Figs. 1 to 4 and 6).

Marchoux[3] suggested that these forms result from conjugation, and Ewing[4] states that while such an explanation appears reasonable, it is without proof, and the more probable explanation is the incomplete fusion of the chromatin in the rosette.

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[3] Marchoux, E., Le paludisme au Sénégal, *Ann. Inst. Pasteur*, 1897, xi, 647.

[4] Ewing, J., Malarial parasitology, *J. Exp. Med.*, 1900–01, v, 482.

As the æstivo-autumnal parasite increases in size, especially if it has developed a thickening of one segment, giving what has been called the "signet-ring" appearance, it is easier to recognize the individual parasites when two or more encircle one mound (Figs. 31 to 34, 36 to 70, 106 to 112, 114 to 118, and 126 to 129).

Craig[5] but follows in the footsteps of the early investigators. He interprets parasites attached to adjoining mounds and parasites attached to one mound as conjugation forms. The theory of conjugation as advanced by him may be summed up in a few words. He states that it occurs "within the infected erythrocytes,"[6] that conjugation "occurs only between two young hyaline forms of the plasmodia, indistinguishable in size and structure,"[7] that it "is completed during the hyaline stage before the formation of pigment,"[8] that the "process occurs in every malarial infection in which quinine has not been given early,"[9] and that it is "the most rational explanation of latency and recurrence in malarial disease."[9] In the same article Craig illustrates[10] two young parasites side by side, a portion of the cytoplasm of one parasite overlying a portion of the cytoplasm of the other parasite. In referring to them he states: "Protoplasmic union is almost complete, and the portions in apposition are beginning to be absorbed." He also pictures[11] two young parasites encircling one corpuscular mound, interpreting the appear-

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[5] Craig, C. F., Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 285, 318.

[6] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 300.

[7] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 304.

[8] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 309.

[9] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 301.

[10] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, Fig. 5.

[11] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, Fig. 7.

ance as "a conjugation form in which the two chromatin masses are distinct and the portions of protoplasm in apposition have become absorbed, resulting in the formation of a large ring-like body with two chromatin masses."[12] In his diagrammatic figures Craig advances the stages in his conjugation theory by bringing the chromatin masses closer and closer together until they are side by side. He finally pictures a large ring-form parasite with one mass of chromatin, which he assumes to be "a form resulting after conjugation is completed."[13]

Premature Division of the Chromatin.

In the adult parasite nuclear division seems to go on more rapidly than cytoplasmic, and it may be completed before cell division takes place. But premature division of the chromatin never takes place in the young parasite.

Certain observers have described what they believed to be a precocious division of the chromatin. Ziemann[14] was at first uncertain whether the appearance was due to two fused parasites or to a precocious division of one nucleus, but finally accepted the latter hypothesis, describing "the separation of one, or rarely two, accessory granules from the original mass in cells infected by single parasites. Sometimes the accessory granule was much smaller than, sometimes nearly as large as, the main granule." And Ewing[15] goes on to say: "All of these appearances I have seen in single parasites, less often in single members of conjugating pairs, and I agree with Ziemann as to their significance." Emin[16] gives examples of two and three parasites encircling one corpuscular mound, and interprets the condition as that of precocious division of the chromatin.

Variation in the size of the chromatin masses of young parasites is frequently observed. It may be only an apparent variation in size, or a breaking up of the chromatin due to technique, or it may be a normal occurrence. The nuclei of young parasites may appear

[12] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 308.

[13] Craig, Studies in the morphology of malarial plasmodia after the administration of quinine, and in intracorpuscular conjugation, *J. Infect. Dis.*, 1910, vii, 318.

[14] Ziemann, H., quoted from Ewing, Malarial parasitology, *J. Exp. Med.*, 1900–01, v, 479.

[15] Ewing, Malarial parasitology, *J. Exp. Med.*, 1900–01, v, 479.

[16] Emin, A., Une variété nouvelle du parasite de Laveran, *Bull. Soc. path. exot.*, 1914, vii, 385; Figs. 3 to 5.

to be larger in certain parts of a film, where the red corpuscles are thinly spread, than they do in the thicker portions. This appearance is due to the flattening of the chromatin mass with consequent enlargement. Irregularity in the size and distribution of chromatin masses in the young parasite is frequently due to technique while spreading the film and illustrates how easily the chromatin may be subdivided. Occasionally young parasites in varying stages of development may occupy one corpuscular mound (Figs. 2, 8, 9, 49, 62, 65, 68 to 70, 112, and 114 to 116). In these instances the cytoplasm may vary in amount and the chromatin mass in size. Where several young parasites are attached to a corpuscle the same variation may be seen (Figs. 26, 73 to 75, 117, and 118). Variations in the size of young parasites are to be expected since all adult parasites do not segment at once in any malarial infection.

Multiple Infection of Red Corpuscles with Crescentic Bodies.

This occurrence (Figs. 77 to 105 and 131 to 140), like multiple infection by young parasites, is always accidental. It has no significance other than the fact that, if several instances are seen in one film, a severe infection is indicated. So far as I know, there have been no theories advanced to explain the occurrence of more than one crescent on a corpuscle. I have seen three crescents on a corpuscle (Fig. 100), but rarely. Two is the number usually seen and I believe that two on a corpuscle are considered a rare occurrence.

There is apparently very little literature referring to two crescents on a corpuscle. Marchiafava and Bignami[17] write: "We have also seen two crescents within the same blood corpuscle, the curved portions being face to face." Manson[18] figures two crescents attached to the one corpuscle, and states: "Very rarely twin or double crescents, that is two crescents in one corpuscle—are encountered." Cropper[19] pictures two crescents on a corpuscle.

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[17] Marchiafava, E., and Bignami, A., Malaria, in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 42.

[18] Manson, P., Tropical diseases: a manual of the diseases of warm climates, London, Paris, New York, and Melbourne, 2nd edition, 1900, 14.

[19] Cropper, J., Phenomenal abundance of parasites in a fatal case of pernicious malaria, *Lancet*, 1908, ii, 16.

In my experience, one of the rarest occurrences in instances of multiple infection of red corpuscles is the presence of a young parasite and a crescent (Figs. 76 and 130). I have seen but three examples of this.

I am convinced that malarial parasites do not conjugate.

Marchiafava and Bignami[20] state: "Not infrequently several young parasites are seen in the same red corpuscle; we have counted up to six or seven, and when they are very close together they may appear to be intimately adherent. . . . . we cannot hold it to have been conclusively demonstrated that the young parasites collected within one red corpuscle become merged together; on the contrary, they follow their own development."

Many of the morphological and biological phases would cease to be obscure if observers realized that malarial parasites are attached to the external surface of red corpuscles.

SUMMARY.

1. Multiple infection of red corpuscles with young parasites is seen in all malarial infections, but it is found most frequently in the æstivo-autumnal infections. The occurrence is accidental and has no significance other than that if the instances are numerous it suggests a heavy infection.

2. In instances of multiple infection the young parasites may be seen to be attached: (*a*) each encircling its own corpuscular mound, giving the typical ring-form picture, or (*b*) two or more encircling one corpuscular mound, giving the appearance of a single ring with two or more masses of chromatin.

3. Certain hypotheses as to the conjugation of malarial parasites have been formulated by observers to explain various instances of multiple infection. I do not believe that conjugation ever occurs. I believe that these hypotheses resulted from observation of certain appearances presented by the attached parasites, as when they are attached so closely together that they may appear to be adherent, or when two or more are attached to one corpuscular mound, giving

[20] Marchiafava and Bignami, Malaria, in Stedman, Twentieth century practice. New York, 1900, xix, 46.

the appearance of a single parasite with more than one mass of chromatin.

4. Certain appearances have also been described as a precocious division of the chromatin masses of young parasites. In these instances the chromatin granules were usually described as varying in size. Such an appearance may be explained as follows: (*a*) two young parasites in varying stages of development may encircle one corpuscular mound, the cytoplasm of one parasite being superimposed over that of the other parasite, giving a picture of a single ring with two unequal masses of chromatin; or (*b*) the variation in the size and number of the chromatin masses may be the result of traumatism, as the nuclei of young parasites are rather easily broken up.

5. Multiple infection of red corpuscles with crescentic bodies is considered rather a rare occurrence. It is always accidental, and if the instances are numerous it means a severe infection.

6. When one accepts the fact that all malarial parasites are attached to the external surface of the red corpuscles, the biological and morphological characteristics of the parasites cease to be obscure.

EXPLANATION OF PLATES.

PLATE 36.

ÆSTIVO-AUTUMNAL PARASITES (TERTIAN PARASITES, FIG. 35).

Magnification, × 1,840.

FIG. 1. Two very young parasites encircling one surface hemoglobin mound. The cytoplasm of one parasite superimposed over that of the other parasite gives the appearance of a single ring with two chromatin masses. One chromatin mass extends beyond the periphery of the corpuscle.

FIG. 2. Examples of two young parasites encircling one surface hemoglobin mound. The chromatin masses of these parasites vary in size.

FIG. 3. Two young parasites encircling a peripheral hemoglobin mound.

FIG. 4. Three young parasites are attached to this corpuscle; two of them encircle one surface hemoglobin mound at x. Pigment granules are seen at o.

FIG. 5. Two young parasites attached to one surface hemoglobin mound. The nuclei of these parasites are shaped to the mound.

FIG. 6. Three young parasites encircling one surface hemoglobin mound. The parasite at x appears to be more advanced in development than the other two.

FIG. 7. Three young parasites encircling one decolorized hemoglobin mound.

FIG. 8. Four young parasites encircling one surface hemoglobin mound. The chromatin masses vary in size. This figure corresponds to Fig. 112.

FIG. 9. Four young parasites encircling one surface hemoglobin mound. The chromatin masses vary in size.

FIG. 10. Five young parasites are attached to this corpuscle; four of them encircle one surface hemoglobin mound. A pigment granule may be seen at o. This figure corresponds to Fig. 113.

FIGS. 11 to 15. These parasites have developed a thickening of one segment. They encircle, in various positions, adjacent hemoglobin mounds. They are attached so closely together that a portion of the cytoplasm of one parasite is overlying a portion of the cytoplasm of the parasite attached to the adjacent mound (corresponding to Mannaberg's conjugation forms). Figs. 11, 13, and 14 show decolorized hemoglobin mounds in connection with but one of the two parasites, suggesting that one of these parasites attached itself to the red corpuscle before the other parasite did. Pigment granules may be seen at o. Fig. 12 corresponds to Fig. 120.

FIG. 16. Two young parasites encircling one peripheral hemoglobin mound. The apex of the mound is not yet decolorized.

FIGS. 17 to 19. Young parasites are shown encircling adjacent decolorized hemoglobin mounds. In Fig. 17 a pigment granule in connection with one parasite may be seen at o.

FIG. 20. At x two young parasites encircle one hemoglobin mound, and at o the two young parasites are attached to adjacent mounds, a portion of the cytoplasm of one overlying a portion of the cytoplasm of the other parasite.

FIGS. 21 to 23. Young parasites attached to adjacent dehemoglobinized corpuscular mounds. In Fig. 21 pigment granules may be seen at o. Fig. 23 shows two parasites encircling one of the decolorized mounds. The two parasites in Fig. 22 appear to be in a similar stage of development to those seen in Fig. 21, and to have done as much damage to the infected corpuscle, yet pigment granules are seen only in connection with the parasites in Fig. 21. These figures correspond to Figs. 121, 119, and 122.

FIG. 24. Four parasites are attached to this corpuscle. There are two decolorized hemoglobin mounds, each encircled by two parasites.

FIG. 25. Five parasites are attached to the corpuscle, two of them to the peripheral mound at x. Note the variation in size of the chromatin masses.

FIG. 26. Five parasites are attached to the corpuscle. Note the variation in the development of these parasites.

FIG. 27. Six parasites are attached to the corpuscle, two to each hemoglobin mound. Two of the mounds are decolorized; the parasites attached to the third and central mound have attached themselves more recently than the other parasites. This figure corresponds to Fig. 124.

FIG. 28. Six parasites are attached to the corpuscle, two to each hemoglobin mound. These mounds are all decolorized and a pigment granule is seen at o.

Fig. 29. Seven parasites are attached to the corpuscle, two to each decolorized mound and one to the mound which has not yet been decolorized. This figure corresponds to Fig. 125.

Fig. 30. Four parasites are attached to the corpuscle, two to one mound, and two to separate and adjacent mounds. This figure corresponds to Fig. 123.

Figs. 31 to 35. Examples of two parasites attached to one surface hemoglobin mound. These parasites give the appearance of developing crescents much more than do the parasites attached to adjoining mounds; but they are only accidentally so attached, and not conjugating. Fig. 35 was taken from a tertian infection. Note the relatively larger size of the chromatin masses of the tertian parasites. Three free parasites may be seen in Fig. 34. Figs. 32 and 34 correspond to Figs. 126 and 127.

Plate 37.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,840.

Fig. 36. Two young parasites freed from a corpuscle which they have destroyed. This conclusion was reached because of the size of the parasites and the presence of a pigment granule at o.

Figs. 37 and 38. Examples of young parasites encircling peripheral hemoglobin mounds. The parasites in Fig. 38 are older than those in Fig. 37.

Fig. 39. Two parasites encircling with their pseudopodia a peripheral hemoglobin mound. These parasites are larger than those seen in Figs. 37 and 38. Pigment granules may be seen at o. This figure corresponds to Fig 107.

Fig. 40. Three very young parasites encircling one large peripheral hemoglobin mound. A pigment granule may be seen at o. This is one of the largest peripheral mounds that I have seen. This figure corresponds to Fig 108.

Fig. 41. At o two young parasites encircle a peripheral hemoglobin mound, while at x two parasites, in a later stage of development, encircle one surface hemoglobin mound. This figure corresponds to Fig. 106.

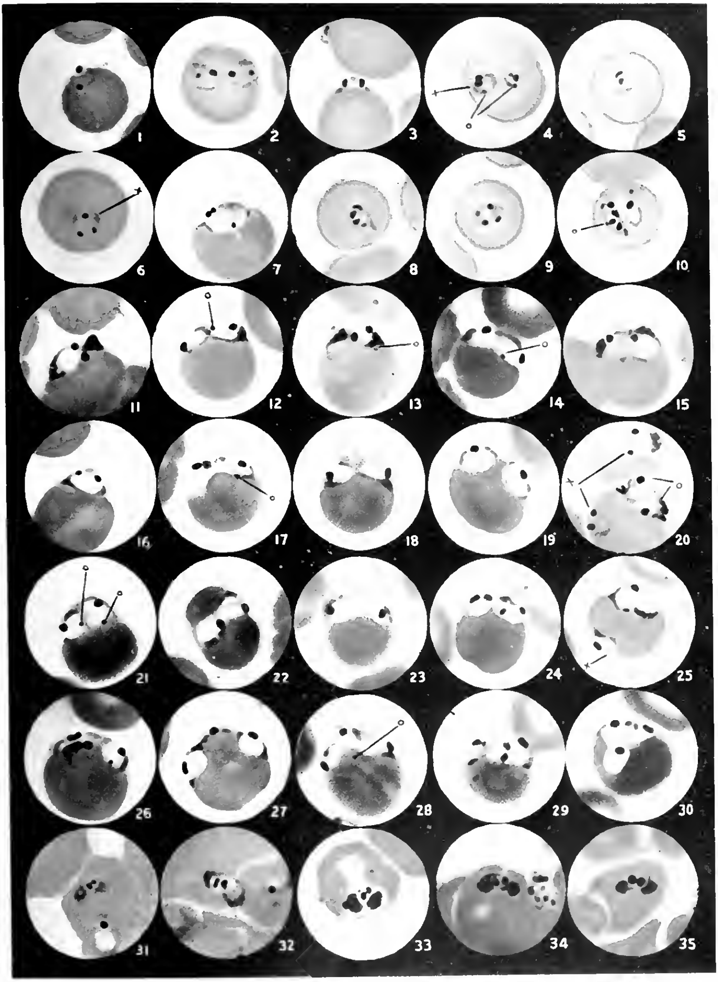
Figs. 42 to 46. Examples of two parasites attached to one surface hemoglobin mound. These parasites have developed a thickening of one segment. A pigment granule is seen in connection with one of the parasites in Fig. 46. In Figs. 42 and 43 the parasites have destroyed the corpuscle to which they were attached and are free in the position they occupied when attached to the corpuscle.

Fig. 47. There are four parasites attached to this corpuscle; three to one mound, and one to an adjoining mound. Note the small size of one of the chromatin masses. A pigment granule is seen at o.

Fig. 48. Two parasites encircling one surface hemoglobin mound. A pigment granule is seen at o. This figure corresponds to Fig. 109.

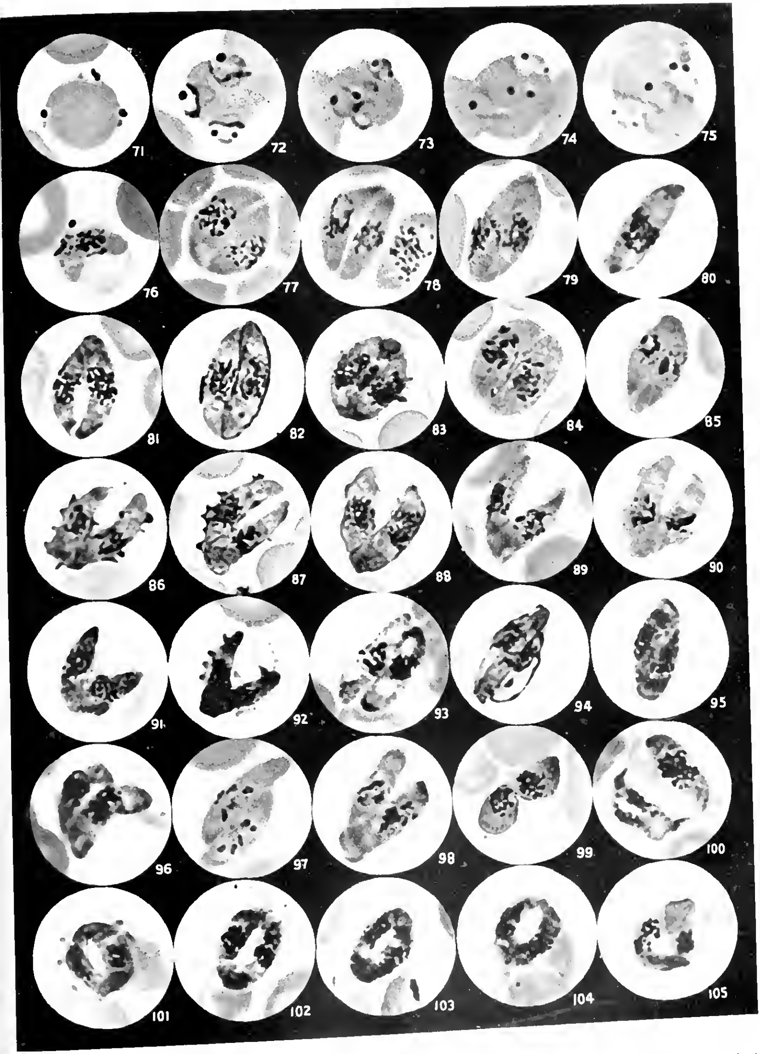
Fig. 49. Three parasites attached to one surface hemoglobin mound. Note the variation in the size of the chromatin masses.

Fig. 50. Two parasites encircling one hemoglobin mound; the body of one of

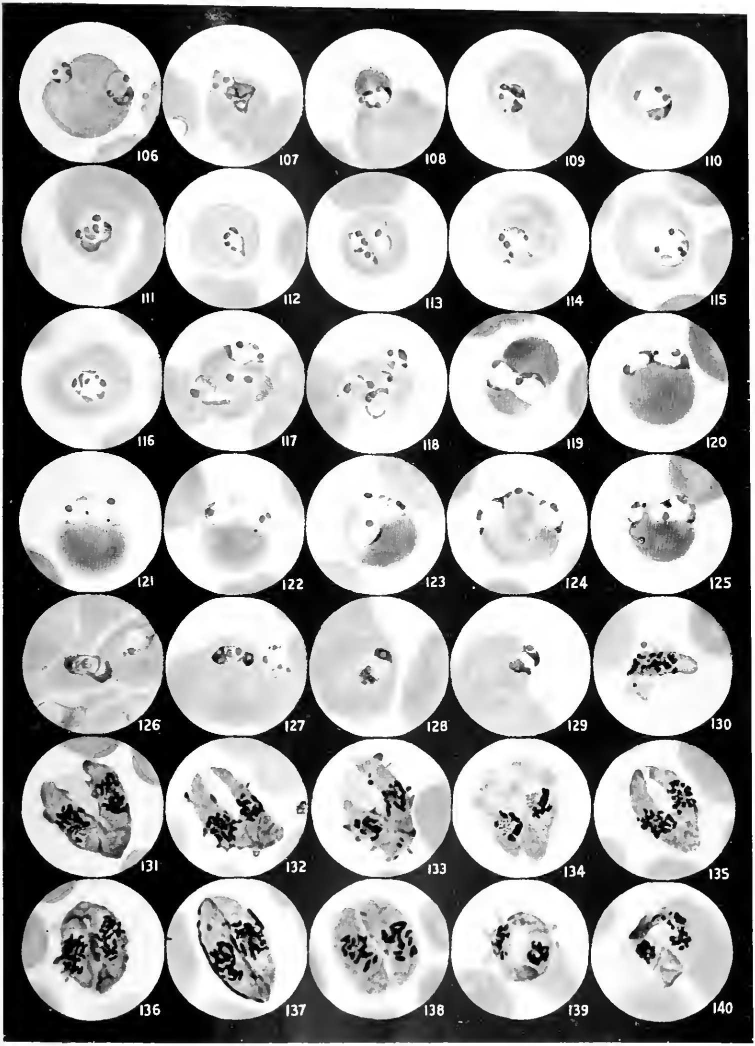


(Lawson: Multiple infection of red corpuscles.)

(Lawson: Multiple infection of red corpuscles.)



(Lawson: Multiple infection of red corpuscles.)



(Lawson: Multiple infection of red corpuscles.)

the parasites rests on the periphery of the corpuscle. A pigment granule is seen at o.

FIGS. 51 and 52. Examples of young parasites encircling one surface hemoglobin mound. These parasites are in similar stages of development; the hemoglobin mound is larger in Fig. 52 than in Fig. 51.

FIGS. 53 and 54. Examples of two parasites in varying stages of development attached to one surface hemoglobin mound. Fig. 53 corresponds to Fig. 110.

FIGS. 55 to 61. Examples of two parasites encircling, in various positions in relation to each other, one surface hemoglobin mound. Fig. 59 shows four parasites, two attached to each surface hemoglobin mound. Figs. 55 and 56 correspond to Figs. 128 and 129.

FIGS. 62 to 67. Examples of three parasites encircling one hemoglobin mound. Note the variation in the size of the chromatin masses in Figs. 62 and 65. In Fig. 65 the hemoglobin mound to which the parasites are attached is easily seen at the periphery of the corpuscle. Fig. 64 corresponds to Fig. 111.

FIGS. 68 and 69. Examples of four parasites encircling one surface hemoglobin mound. Note the variation in size of the chromatin masses. These figures correspond to Figs. 114 and 115.

FIG. 70. Five young parasites encircling one surface hemoglobin mound. There is quite a variation in the size of the chromatin masses. This figure corresponds to Fig. 116.

PLATE 38.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,840.

FIGS. 71 to 75. Instances of multiple infection of red corpuscles by young parasites (four to seven). Each chromatin mass corresponds to one or two parasites. In Fig. 71 the parasites are very young and have attached themselves to the periphery of the corpuscle, two at the top, two at the right, and two at the left. Fig. 72 shows four parasites, Fig. 73, seven parasites, and Figs. 74 and 75, five parasites. In Figs. 72 to 75 note the variation in size of the chromatin masses. Figs. 73 and 74 correspond to Figs. 118 and 117.

FIG. 76. A crescent and a young parasite attached to the same red corpuscle. This figure corresponds to Fig. 130.

FIGS. 77 to 98 and 101 to 105. Examples of two crescents attached to one red corpuscle. In Figs. 83, 86 to 88, 90 to 93, 96, and 101, the mounds of hemoglobin substance to which the crescents have attached themselves are well shown. In Fig. 93 the hemoglobin mounds have been dehemoglobinized by the parasites. In Fig. 94 a pseudopodium arising from the cytoplasm of the parasite is shown in the form of a large loop.

Figs. 81, 82, 83, 84, 86, 87, 88, 90, 101, and 105 correspond to Figs. 135, 137, 136, 138, 133, 132, 131, 134, 139, and 140.

FIG. 99. This figure may be variously interpreted. It may be two contracted crescents, it may be a crescent twisted on itself, or it may be a segmenting crescent.

FIG. 100. Three crescents attached to one corpuscle. This showed better in the stained specimen than it does in the photograph.

PLATE 39.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,840.

FIG. 106. Four young æstivo-autumnal parasites attached to a corpuscle; two encircle a peripheral hemoglobin mound, and two, in a more advanced stage of development, encircle a surface hemoglobin mound. This figure corresponds to Fig. 41.

FIG. 107. Two young æstivo-autumnal parasites, with abundant cytoplasm, encircling a peripheral hemoglobin mound. Two pigment granules are seen in connection with one of the parasites. This figure corresponds to Fig. 39.

FIG. 108. Three very small æstivo-autumnal parasites encircling a very large peripheral hemoglobin mound. This figure corresponds to Fig. 40.

FIG. 109. Two young æstivo-autumnal parasites, in similar stages of development, encircling one surface hemoglobin mound. A pigment granule is seen in connection with one of the parasites. It is easily seen that these are individual parasites. This figure corresponds to Fig. 48.

FIG. 110. Two young æstivo-autumnal parasites, in varying stages of development, encircling one surface hemoglobin mound. This figure corresponds to Fig. 53.

FIG. 111. Three young æstivo-autumnal parasites, in similar stages of development, encircling one surface hemoglobin mound. This figure corresponds to Fig. 64.

FIG. 112. Four very young æstivo-autumnal parasites encircling one surface hemoglobin mound. Note the variation in size of the chromatin masses. This figure corresponds to Fig. 8.

FIG. 113. Five parasites are attached to this corpuscle. Four of them encircle one surface hemoglobin mound; a pigment granule is seen in connection with one of these. This figure corresponds to Fig. 10.

FIG. 114. Four young æstivo-autumnal parasites encircling one surface hemoglobin mound. These parasites are in varying stages of development. Note the variation in size of the chromatin masses. This figure corresponds to Fig. 68.

FIG. 115. Four young æstivo-autumnal parasites, in varying stages of development, encircling one surface hemoglobin mound. One of the chromatin masses is slightly smaller than the others. This figure corresponds to Fig. 69.

FIG. 116. Five young æstivo-autumnal parasites, in varying stages of development, attached to one surface hemoglobin mound. Note that one of the chromatin masses is distorted. This figure corresponds to Fig. 70.

FIG. 117. Five young æstivo-autumnal parasites attached to surface hemoglobin mounds. Note that these parasites are not all in similar stages of development. This figure corresponds to Fig. 74.

FIG. 118. Seven young æstivo-autumnal parasites attached to surface hemoglobin mounds. Note the small size of one of these parasites. This figure corresponds to Fig. 73.

FIG. 119. Two young æstivo-autumnal parasites attached to adjacent decolorized hemoglobin mounds. This figure corresponds to Fig. 22.

FIG. 120. Two young æstivo-autumnal parasites encircling two decolorized hemoglobin mounds. A granule of pigment may be seen in connection with one of these parasites. A portion of the cytoplasm of one parasite is seen to overlie a portion of the cytoplasm of the parasite attached to the adjacent hemoglobin mound. This figure corresponds to Fig. 12.

FIG. 121. Two young æstivo-autumnal parasites attached to adjoining hemoglobin mounds. The mounds are decolorized and a pigment granule is seen in connection with each parasite. This figure corresponds to Fig. 21.

FIG. 122. Three young æstivo-autumnal parasites are attached to this corpuscle. Two encircle the decolorized hemoglobin mound at the right (a pigment granule is seen in connection with them), and one encircles the decolorized mound at the left. This figure corresponds to Fig. 23.

FIG. 123. Four young æstivo-autumnal parasites are attached to this corpuscle. Two of them encircle one decolorized hemoglobin mound. This figure corresponds to Fig. 30.

FIG. 124. Six young æstivo-autumnal parasites are attached to this corpuscle, two attached to each hemoglobin mound. Two of the hemoglobin mounds have been decolorized by the action of the parasites. This figure corresponds to Fig. 27.

FIG. 125. Seven young æstivo-autumnal parasites are attached to this corpuscle. Two parasites encircle each of the three decolorized hemoglobin mounds; pigment granules are seen in connection with these parasites. This figure corresponds to Fig. 29.

FIG. 126. Two young æstivo-autumnal parasites attached to one surface hemoglobin mound. This figure corresponds to Fig. 32.

FIG. 127. Two young æstivo-autumnal parasites attached to one surface hemoglobin mound. To the right may be seen three young parasites free. This figure corresponds to Fig. 34.

FIGS. 128 and 129. Examples of two young æstivo-autumnal parasites attached to one surface hemoglobin mound. One of the two parasites in Fig. 129 rests on the periphery of the corpuscle. These figures correspond to Figs. 55 and 56.

FIG. 130. A young æstivo-autumnal parasite and a crescent attached to the same red corpuscle. This figure corresponds to Fig. 76.

FIGS. 131 to 140. Examples of two crescents on one corpuscle. In Figs. 131 to 136 and 139, the mounds of hemoglobin substance to which the crescents are attached are well shown. These figures correspond to Figs. 88, 87, 86, 90, 81, 83, 82, 84, 101, and 105.

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